The EMBO Journal (2008) 27, 589–605 | © 2008 European Molecular Biology Organization | All Rights Reserved 0261-4189/08
www.embojournal.org

THE EMBO JOURNAL

Focus Quality Control

DNA-damage repair; the good, the bad, and the ugly

Razqallah Hakem[1,2,*]

[1]Department of Medical Biophysics, Ontario Cancer Institute/UHN, University of Toronto, Toronto, Ontario, Canada and [2]Department of Immunology, Ontario Cancer Institute/UHN, University of Toronto, Toronto, Ontario, Canada

Organisms have developed several DNA-repair pathways as well as DNA-damage checkpoints to cope with the frequent challenge of endogenous and exogenous DNA insults. In the absence or impairment of such repair or checkpoint mechanisms, the genomic integrity of the organism is often compromised. This review will focus on the functional consequences of impaired DNA-repair pathways. Although each pathway is addressed individually, it is essential to note that cross talk exists between repair pathways, and that there are instances in which a DNA-repair protein is involved in more than one pathway. It is also important to integrate DNA-repair process with DNA-damage checkpoints and cell survival, to gain a better understanding of the consequences of compromised DNA repair at both cellular and organismic levels. Functional consequences associated with impaired DNA repair include embryonic lethality, shortened life span, rapid ageing, impaired growth, and a variety of syndromes, including a pronounced manifestation of cancer.

The EMBO Journal (2008) **27,** 589–605. doi:10.1038/emboj.2008.15
Subject Categories: molecular biology of disease
Keywords: cancer; DNA repair; mouse models; syndromes

Introduction

Organisms have evolved to efficiently respond to DNA insults that result from either endogenous sources (cellular metabolic processes) or exogenous sources (environmental factors). Endogenous sources of DNA damage include hydrolysis, oxidation, alkylation, and mismatch of DNA bases; sources for exogenous DNA damage include ionizing radiation (IR), ultraviolet (UV) radiation, and various chemicals agents. At the cellular level, damaged DNA that is not properly repaired can lead to genomic instability, apoptosis, or senescence, which can greatly affect the organism's development and ageing process. More importantly, loss of genomic integrity predisposes the organism to immunodeficiency, neurological disorders, and cancer (O'Driscoll and Jeggo, 2006; Subba Rao, 2007; Thoms *et al*, 2007). Therefore, it is essential for cells to efficiently respond to DNA damage through coordinated and integrated DNA-damage checkpoints and repair pathways.

*Corresponding author. Department of Medical Biophysics, Ontario Cancer Institute/UHN, University of Toronto, 610 University Avenue PMH, Room 10-622, Toronto, Ontario, Canada M5G 2M9.
Tel.: +1 416 946 2398/4501; ext: 5661; Fax: +1 416 946 2984;
E-mail: rhakem@uhnres.utoronto.ca

Received: 31 October 2007; accepted: 16 January 2008

DNA-damage checkpoints

The mechanisms of DNA-damage checkpoints are best understood during their responses to double-strand breaks (DSBs). Initiation of these checkpoints is dependent on the transient recruitment of the MRE11/RAD50/NBS1 (MRN) complex at DSB sites, followed by the recruitment/activation of ataxia–telangiectasia mutated (ATM) a member of the family of phosphoinositide-3-kinase-related kinases (PIKKs) (Su, 2006). In addition, two other PIKKs, DNA-dependent protein kinase (DNA–PK) and ATR (ATM and Rad3 related), are also activated and involved in the response to DSBs. However, the primary function of ATR is the initiation of DNA-damage response to stalled replication forks (RFs) (Su, 2006). ATM, ATR, and DNA–PK phosphorylate various targets that contribute to the overall DNA damage response. Therefore, within minutes of DSB formation, active ATM phosphorylates different proteins that are essential for DNA-damage response and repair. An example includes the histone H2AX that, following its phosphorylation at the site of DNA damage by ATM, DNA–PK, or ATR (γH2AX), recruits other proteins and initiates the chromatin-remodelling process that is essential for the repair of damaged DNA. Other proteins recruited to sites of DSBs include MDC1, 53BP1, and BRCA1, all of which are ATM substrates and mediators in DNA-damage response. The MRN complex-mediated resection of DSBs is followed by single-stranded DNA coating with replication protein A (RPA), which serves to recruit ATR and its binding partner ATRIP, and subsequent ATR-dependent phosphorylation of clapsin, Rad17, BRCA1, and others (Su, 2006).

ATM and ATR are essential for the G1/S, intra-S-phase, and G2/M DNA-damage checkpoints, and are critical for the maintenance of genomic integrity. Defects in either ATM or ATR have been associated with human syndromes. *ATM* mutations are associated with the human ataxia–telangiectasia (AT), an autosomal recessive disorder characterized by cerebellar ataxia, progressive mental retardation, impaired immune functions, neurological problems, and malignancies (O'Driscoll and Jeggo, 2006). At the cellular level, AT phenotypes include chromosomal breakage and IR sensitivity. Similarly, *ATR* mutations predispose individuals to Seckel syndrome, a very rare autosomal recessive human disorder

characterized by growth and mental retardation, as well as microcephaly (O'Driscoll and Jeggo, 2006). Spontaneous and IR-induced genomic instability and immunological defects have also been observed in Seckel syndrome patients. In contrast to ATM and ATR, no human syndrome has yet been associated with defective DNA–PK. However, studies of mouse models have linked mutations of *DNA–PK* to severe immunodeficiency (see section Non-homologous end joining repair pathway).

Activated ATM and ATR mediate the phosphorylation and subsequent activation of Chk2 and Chk1, respectively; this process is necessary in the induction of phosphorylation of CDC25A, marking it for proteosomal degradation (Su, 2006). The consequential loss of CDC25A results in G1/S arrest, due to the inefficient loading of CDC45 at the origin of replication. In addition, activated ATM, ATR, DNA–PK, Chk2, and Chk1 all aid in the phosphorylation and activation of p53, a key player in DNA-damage checkpoints. Activated p53 transactivates p21, which inhibits two G1/S-promoting cyclin-dependent kinases (CDKs), CDK2 and CDK4. This leads to sustained G1 arrest, which ultimately hampers the replication of damaged DNA.

The intra-S-phase checkpoint serves to arrest DNA synthesis during S phase of cells with damaged DNA (Su, 2006). In these cells, CDC25A phosphorylation, mediated by Chk2 or Chk1, leads to its degradation and the subsequent inactivation of the S-phase cyclin E/CDK2 complex. Consequently, these events prevent the loading of CDC45 at the origin of replication and result in intra-S-phase arrest. It has been reported that other proteins, including Nijmegen breakage syndrome 1 (NBS1), BRCA1, SMC1, 53BP1, and MDC1, all contribute to the intra-S-phase checkpoint.

The activation of the G2/M DNA-damage checkpoint prevents mitotic entry of the damaged cells (Su, 2006). This checkpoint is mediated by the dual-specificity phosphatase CDC25C, as well as p53. In normal conditions, CDC25C dephosphorylates CDC2, allowing the CDC2–cyclin B kinase to facilitate entry into mitosis. However, phosphorylation of CDC25C by Chk2 or Chk1, initiates its binding with 14-3-3, which leads to its cytoplasmic sequestration away from its substrate, thus preventing mitotic entry. p53 also contributes to the G2/M checkpoint through its transactivation of p21 and 14-3-3. P21 effectively blocks the phosphorylation of CDC2, initiating the onset of the G2/M cell-cycle arrest. 14-3-3 sequesters CDC25C in the cytoplasm and promotes the activation of Wee1, a tyrosine kinase that negatively regulates CDC2, thus blocking entry into mitosis.

The activation of DNA-damage checkpoints enforces the growth arrest of damaged cells and allows the DNA-repair mechanisms to mend the damaged DNA. Once repair is completed, cells are able to exit the checkpoints and resume their cell-cycle progression and functions. However, unsuccessful DNA repair leads to p53-dependent apoptosis (Chipuk and Green, 2006), in addition to senescence (Collado *et al*, 2007).

Defects of DNA-damage checkpoints, similar to impaired DNA-damage repair, promote genomic instability and predispose individuals to immunodeficiency, neurological defects, and cancer (Niida and Nakanishi, 2006). Although important advances have been made in understanding the cellular mechanisms behind the initiation and maintenance of checkpoints, the mechanisms that control checkpoints exit, as well as how the cell decides survival, death, or senescence, require further investigation.

Defects associated with DNA-damage repair pathways

Different DNA-repair pathways exist and perform major roles at both cellular and organismic levels. These pathways include (1) the direct reversal pathway, (2) the mismatch repair (MMR) pathway, (3) the nucleotide excision repair (NER) pathway, (4) the base excision repair (BER) pathway, (5) the homologous recombination (HR) pathway, and (6) the non-homologous end joining (NHEJ) pathway (Figure 1). The mechanisms for these pathways will not be discussed in detail in this review; instead we will focus on the functional consequences associated with their defects.

Direct reversal of DNA damage

In contrast to other DNA-damage repair pathways, direct reversal of DNA damage is not a multistep process and does not involve multiple proteins (Sedgwick *et al*, 2007). Furthermore, unlike excision repair, direct reversal of DNA damage does not require the excision of the damaged bases. An example of a DNA lesion that is repaired by direct reversal is the O^6-alkylguanine. Alkylating agents can transfer methyl or ethyl groups to a guanine, thereby modifying the base and interfering with its pairing with cytosine during DNA replication. The cytotoxic and mutagenic O^6 alkyl adduct in DNA is repaired by direct reversal, which is mediated by the enzyme Ada in *Escherichia coli* (*E. coli*) and the mammalian O^6-methylguanine-DNA methyltransferase (MGMT). MGMT, also known as AGT, removes the DNA adducts by transferring the alkyl group from the oxygen in the DNA to a cysteine residue in its active site. This reaction leads to the reversal of the base damage; however, the alkylation of MGMT leads to its inactivation and subsequent ubiquitination and proteosomal degradation. MGMT has attracted a great deal of attention, as certain anticancer chemotherapeutic drugs produce O^6-alkylguanine, further supporting its role in modulating the therapeutic response of tumors to these drugs. Mouse models for *Mgmt* inactivation have been generated (Tsuzuki *et al*, 1996b; Glassner *et al*, 1999). These mutants were viable and

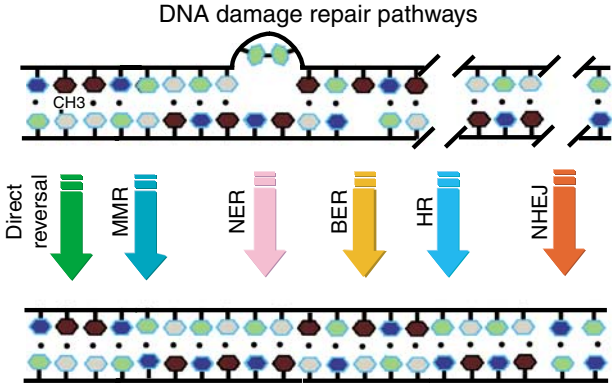

Figure 1 DNA-repair pathways. Several DNA-repair pathways exist and deal with various types of DNA insults. These pathways include (1) the direct reversal pathway, (2) the MMR pathway, (3) the NER pathway, (4) the BER pathway, (5) the HR pathway, and (6) the NHEJ pathway.

Table I Examples of mouse models for direct reversal

Genotype	Developmental defects	Fertility defects	Spontaneous tumorigenesis	Induced tumorigenesis	References
Mgmt−/−	None	None	Not affected	Increased DMNA-induced lung and liver cancer in females	(Tsuzuki *et al*, 1996a, b; Iwakuma *et al*, 1997; Glassner *et al*, 1999)
Abh2−/−	None	None	Not affected	Not tested	(Ringvoll *et al*, 2006)
Abh3−/−					
Abh2−/−Abh3−/−					

showed no increase in spontaneous tumorigenesis (Table I). However, *Mgmt* homozygous mice and cells were highly sensitive to chemotherapeutic alkylating agents such as methylnitrosourea. *Mgmt* homozygous mutant females, but not males, developed larger numbers of dimethylnitrosamine-induced liver and lung tumors compared with controls (Iwakuma *et al*, 1997). Additionally, transgenic mice over-expressing human MGMT or *E. coli* Ada have also been generated. In response to alkylating carcinogens that produce O^6-alkylguanine in DNA, these transgenic mice demonstrated a significantly reduced susceptibility to developing cancers, including thymomas (Dumenco *et al*, 1993), liver tumors (Nakatsuru *et al*, 1993), and skin tumors (Becker *et al*, 1997).

AlkB is another enzyme that mediates direct DNA damage reversal in *E. coli*. This dioxygenase is involved in the repair of alkylation damage, particularly 1-methyladenine (1meA) and 3-methylcytosine (3meC). Two mammalian AlkB homologues, ABH2 and ABH3, have been shown to possess DNA-repair functions similar to the bacterial AlkB (Duncan *et al*, 2002; Sedgwick *et al*, 2007). Similar to AlkB, ABH2 and ABH3 have the ability to repair 1meA and 3meC residues. However, whereas ABH2 prefers double-stranded DNA, ABH3 and AlkB favour single-stranded DNA and RNA (Aas *et al*, 2003; Falnes *et al*, 2004). Further insight into the function of the mammalian ABH2 and ABH3 came from studies of mice carrying targeted mutations of these genes. Mice deficient in Abh2, Abh3, or both, were viable (Ringvoll *et al*, 2006). $Abh2^{-/-}$, but not $Abh3^{-/-}$, mice showed age-dependent accumulation of 1meA in their genomic DNA. As in *AlkB* mutants in *E. coli*, mouse embryonic fibroblasts (MEFs) deficient in Abh2 were hypersensitive to methyl methane-sulfonate (MMS) treatment. However, mice deficient in Abh2 or Abh3 did not show increased spontaneous cancer development (Table I). Further studies are required to assess the role of these dioxygenases, and other AlkB homologues, in alkylation damage-induced cancer.

These examples of direct DNA-damage reversal mediated by MGMT/Ada or ABH/AlkB demonstrate the conserved role of this mechanism in DNA repair. In addition, increased tumorigenesis of *Mgmt* mutants, together with the resistance of MGMT transgenic mice to alkylating carcinogens that produce O^6-alkylguanine, further demonstrate the important role that direct reversal plays in cancer.

The MMR pathway

The MMR pathway plays an important role in both prokaryotes and eukaryotes in repairing mismatches, which are small insertions and deletions that take place during DNA replication (Figure 1; Jiricny, 2006). Failure of MMR commonly results in microsatellite instability (MSI). Several

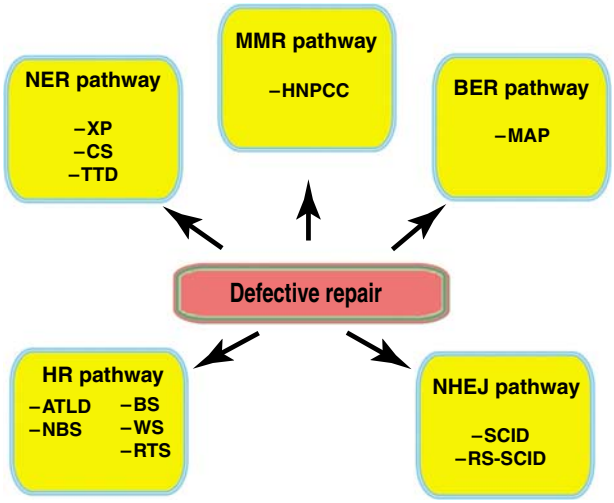

Figure 2 Examples of human syndromes and disorders associated with defective DNA-damage repair. Impaired MMR pathway leads to the hereditary HNPCC. Mutations of certain human NER genes have been associated with syndromes and disorders including the XP, CS, and TTD. MAP, a rare disorder, has been shown associated with mutations of the BER gene *MUTYH*. Various human syndromes and disorders have been associated with defects of the HR pathway. They include ATLD, NBS, BS, WS and RTS. Mutations of certain human genes involved in NHEJ lead to the SCID or RS-SCID.

homologues of the bacterial MMR genes *MutS* and *MutL* have been identified in yeast and mammals.

The importance of the MMR pathway became evident upon identification of mutations in certain human MMR genes in hereditary non-polyposis colorectal cancer (HNPCC), a highly penetrant autosomal dominant cancer syndrome (Figure 2; Vasen *et al*, 2007). HNPCC, also known as Lynch syndrome, is characterized by early-onset colorectal cancer, with elevated levels of MSI in the tumors. Individuals with HNPCC have an approximate 80% lifetime risk for colorectal cancer, and are also predisposed to the development of endometrial, ovarian, gastric, and other types of malignancies.

Approximately 70–80% of germline mutations identified in HNPCC families are mutations in *MLH1* or *MSH2*, whereas mutations in *MSH6* are found in approximately 10% of HNPCC families (Peltomaki and Vasen, 1997). Germline mutations in other human MMR genes, including *PMS1*, *PMS2*, *MLH3*, and *exonuclease 1* (*EXO1*), have also been found in HNPCC families; however, they occur at a much lower frequency (Vasen *et al*, 2007). In addition, inactivation of *MLH1* by mutations at the promoter or coding sequences, or by promoter methylation, has been identified in sporadic colorectal tumors (Kane *et al*, 1997; Veigl *et al*, 1998). Recent

studies, although very limited, have identified rare patients with homozygous germline mutations for *MLH1*, *MSH2*, *MSH6*, or *PMS2* (Felton *et al*, 2007). Typically, these individuals have a reduced life span and, in contrast to heterozygous MMR individuals, tend to develop juvenile haematological malignancies and brain cancer.

In yeast, Msh2 forms heterodimers with Msh3 and Msh6, proteins that bind DNA mismatches and initiate the MMR process. In *Saccharomyces cerevisiae* (*S. cerevisiae*), *Msh2*, *Msh3*, and *Msh6* mutants are viable (Marsischky *et al*, 1996). Both *Msh2* and *Msh6 S. cerevisiae* mutants show high frequencies of base substitution, whereas only *Msh2* mutants exhibit high frameshift mutations. *Msh3* mutations in *S. cerevisiae* result in low rates of frameshift mutations. However, on *Msh6*-mutant background, synergistic effects of the dual mutations have been observed, including increased MSI and mutability similar to *Msh2* mutants.

Mutant mice for *MutS* and *MutL* MMR homologues have also been generated using gene targeting (Table II). Mutant mice for the *MutS* homologues include *Msh2*, *Msh3*, *Msh4*, *Msh5*, and *Msh6*. Mice carrying homozygous mutations for *Msh4* or *Msh5* did not exhibit cancer phenotypes; however, males and females were infertile, consistent with the role of *MutS* homologues in processing meiotic recombination intermediates (de Vries *et al*, 1999; Edelmann *et al*, 1999). In contrast, homozygous mutants for *Msh2* (de Wind *et al*, 1995; Reitmair *et al*, 1995), *Msh3* (Edelmann *et al*, 2000), and *Msh6* (Edelmann *et al*, 1997) have increased risk for developing cancers such as lymphoma, gastrointestinal, and skin cancer.

Mutant mice for *MutL* homologues include $Pms1^{-/-}$, $Pms2^{-/-}$, $Mlh1^{-/-}$, and $Mlh3^{-/-}$ mice (Table II). These mutants are viable; however, males and females deficient in Mlh1 (Baker *et al*, 1996; Edelmann *et al*, 1996) or Mlh3 (Lipkin *et al*, 2002) and males deficient in Pms2 (Baker *et al*, 1995) are sterile, demonstrating a requirement for these proteins during meiosis. In addition, mouse *MutL* homologues are differentially required for cancer suppression. $Pms1^{-/-}$ mice do not show any increased risk for cancer (Prolla *et al*, 1998), whereas $Mlh1^{-/-}$ (Prolla *et al*, 1998; Chen *et al*, 2005) and $Mlh3^{-/-}$ mice (Chen *et al*, 2005) are predisposed to developing lymphomas and gastrointestinal tumors. Similarly, *Pms2*-null mutants (Prolla *et al*, 1998; Chen *et al*, 2005) are prone for lymphoma development.

EXOI physically interacts with MSH2, MSH3, and MLH1, and is involved in the excision of mismatched bases in DNA (Tishkoff *et al*, 1997; Schmutte *et al*, 2001). Mutant mice for *ExoI* have impaired MMR, accumulate MSI, and exhibit a greater risk for developing lymphomas (Wei *et al*, 2003). These mutants also have meiotic defects and are sterile, demonstrating the requirement of ExoI in meiosis.

Double mutant mice carrying dual mutations of different MMR genes have also been reported. For example, *Msh3*-mutant mice develop cancer with low frequency and at a later age, whereas $Msh3^{-/-}Msh6^{-/-}$ mice (Edelmann *et al*, 2000) die prematurely and develop tumors including lymphomas, gastrointestinal, and skin tumors. This phenotypic outcome is similar to that of $Msh2^{-/-}$ or $Mlh1^{-/-}$ mice that are the most cancer-prone MMR mutants, as half of these mutants die around 6 months of age. This cooperation between mutations of *Msh2* and *Msh6* in mice is reminiscent of their collaboration in the maintenance of genomic integrity of *S. cerevisiae*.

Immunoglobulin (Ig) diversification, an essential process for immunity, involves somatic hypermutation (SHM) of the Ig genes, as well as VDJ recombination and class-switch recombination (CSR), two processes mediated by NHEJ (Maizels, 2005). Interestingly, studies of the various MMR-mutant strains have implicated a role for this pathway in SHM and CSR. Thus, *Msh2*, *Msh6*, and *ExoI*, but not *Msh3*-mutant mice, have reduced CSR and SHM (Rada *et al*, 1998; Wiesendanger *et al*, 2000; Bardwell *et al*, 2004; Li *et al*, 2004).

Table II Examples of mouse models for the MMR pathway

Genotype	Developmental defects	Fertility defects	Spontaneous tumorigenesis	References
Msh2−/−	None	None	High frequency and early onset of lymphomas, gastrointestinal, and skin cancer	(de Wind *et al*, 1995; Reitmair *et al*, 1995)
Msh3−/−	None	None	Low frequency and late onset of lymphomas, gastrointestinal, and skin cancer	(Edelmann *et al*, 2000)
Msh4−/−	None	Infertile	Not affected	(Kneitz *et al*, 2000)
Msh5−/−	None	Infertile	Not affected	(de Vries *et al*, 1999; Edelmann *et al*, 1999)
Msh6−/−	None	Unaffected	Lymphoma, gastrointestinal, and skin cancer	(Edelmann *et al*, 1997)
Msh3−/−Msh6−/−	None	None	Higher frequency of lymphomas, gastrointestinal, and skin tumours compared to single mutants	(Edelmann *et al*, 2000)
Pms1−/−	None	None	Not affected	(Prolla *et al*, 1998)
Pms2−/−	None	Male infertility	Lymphomas	(Baker *et al*, 1995; Prolla *et al*, 1998; Chen *et al*, 2005)
Mlh1−/−	None	Infertile	High frequency and early onset of lymphomas and gastrointestinal tumours	(Baker *et al*, 1996; Edelmann *et al*, 1996; Prolla *et al*, 1998; Chen *et al*, 2005)
Mlh3−/−	None	Infertile	Lymphomas and gastrointestinal tumours	(Lipkin *et al*, 2002; Chen *et al*, 2005)
ExoI−/−	None	Infertile	Lymphomas	(Wei *et al*, 2003)

Whereas most HNPCC human individuals carry heterozygous germline mutations of MMR genes, which predisposes them to cancer, mice heterozygous for MMR mutations do not appear to have an increased risk for developing cancer. This difference is not specific for MMR mutations, as heterozygous mutations in certain genes involved in other DNA-damage repair pathways are also able to predispose humans, but not mice, to cancer. The reasons for these differences remain unknown, although species differences in the DNA-damage repair pathways, metabolism, or life span could contribute to these observed human–mouse discrepancies. Despite these differences, mouse models have significantly improved our understanding of the MMR and other repair mechanisms, and their roles in preserving genomic integrity and suppressing cancer.

The NER pathway

The NER pathway is a multistep process that serves to repair a variety of DNA damage, including DNA lesions caused by UV radiation, mutagenic chemicals, or chemotherapeutic drugs that form bulky DNA adducts (Figure 1; Leibeling *et al*, 2006). Over 30 different proteins are involved in the mammalian NER, whereas only three proteins (UvrA, UvrB, and UvrC) are required by prokaryotes (Truglio *et al*, 2006).

Two NER sub-pathways that have been identified are as follows: the global genome NER (GG-NER) that detects and removes lesions throughout the genome, and the transcription-coupled NER (TC-NER), which repairs actively transcribed genes. NER begins with the recognition of the DNA lesion, followed by incisions at sites flanking the DNA lesion, and culminates in the removal of the oligonucleotide containing the DNA lesion. Ligation of a newly synthesized oligonucleotide, complementary to the pre-existing strand, serves to fill the gap, thus ending the NER process. GG-NER and TC-NER involve several common proteins and proceed through the same repair steps, except during recognition of the DNA lesion. In GG-NER this recognition involves the XPC–RAD23B and DDB1–DDB2/XPE proteins, whereas recognition in TC-NER is mediated by cockayne syndrome group A (CSA) (ERCC8) and CSB (ERCC6). NER has attracted a great deal of attention due to its role in three rare human syndromes characterized by increased cancer frequencies, neurodegeneration and ageing (Figure 2). These syndromes are xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD) (Thoms *et al*, 2007).

XP individuals show extremely severe skin sensitivity to short intervals of sun exposure and most develop freckles at an early age. In addition, XP individuals may exhibit eye damage as they suffer chronic UV-induced conjunctivitis and keratitis as a consequence of continual sun exposure. XP individuals have greater than 1000-fold increased skin cancer risk, which first appears at an average age of 10 years. Approximately 20% of XP individuals also develop neurological abnormalities. XP is caused by mutations of the NER gene *XPA*, *XPB* (*ERCC3*), *XPC*, *XPD* (*ERCC2*), *XPE* (*DDB2*), or *XPF; XPG*. Whereas XP individuals carrying mutations of *XPC* or *XPE* (*DDB2*) are only deficient in GG-NER, the remaining XP individuals are deficient in both GG-NER and TC-NER (Thoms *et al*, 2007).

CS is a very rare human autosomal recessive inherited genetic disease (Thoms *et al*, 2007). Similar to XP individuals, CS individuals suffer excessive sun sensitivity, but without increased predisposition for skin cancer. Common CS symptoms include growth retardation (dwarfism), progressive cognitive impairment, and ophthalmologic disorders such as cataracts or retinitis pigmentosa. CS individuals typically die in the first or second decade of life. CS is caused by mutations of either CSA or CSB, two proteins essential for DNA-damage recognition and initiation of TC-NER. Therefore, although CS individuals are deficient in TC-NER, they remain proficient in GG-NER.

TTD is a rare human autosomal recessive disorder associated with defective NER; the most severe cases are associated with mutations in the *XPB* or *XPD* genes. Clinical characteristics of TTD include brittle hair and nails, dwarfism, and ataxia (Thoms *et al*, 2007). In addition, half of TTD individuals exhibit sensitivity to sunlight; however, skin cancer predisposition has not been linked to this syndrome.

Several mouse models for NER mutations have been generated (Table III). Homozygous mutants for *XP* genes are viable (Nakane *et al*, 1995; Sands *et al*, 1995; Harada *et al*, 1999; Itoh *et al*, 2004; Tian *et al*, 2004a, b; Yoon *et al*, 2005), with the exception of the pre-implantation embryonic lethality of *Xpd* mutants (de Boer *et al*, 1998b). Xpd^{R722W}-mutant mice carrying an amino-acid substitution that mimics a human *XPD* allele associated with TTD have been generated (de Boer *et al*, 1998a). Similarly, Xpd^{G602D}-mutant mice carrying a substitution at amino acid 602 have been generated to mimic the human combined XP/CS (Andressoo *et al*, 2006). Both Xpd^{R722W} and Xpd^{G602D} mutants have been proven viable and reproduced some of the characteristics of individuals that carry these *XPD* mutations.

With the exception of *Xpe* (*Ddb2*) mutants (Itoh *et al*, 2004; Yoon *et al*, 2005), homozygous mutant mouse cells for *Xpa* (Nakane *et al*, 1995), *Xpc* (Sands *et al*, 1995), *Xpf* (Tian *et al*, 2004b), *Xpg* (Harada *et al*, 1999; Tian *et al*, 2004a), Xpd^{R722W} (de Boer *et al*, 1998a), or Xpd^{G602D} (Andressoo *et al*, 2006) were all UV sensitive, correlating to the results obtained from human mutant cells. Increased predisposition for UV-induced skin cancer was observed with *Xpa*, *Xpc*, Xpd^{R722W}, Xpd^{G602D}, and *Xpe* (*Ddb2*)-mutant mice (Nakane *et al*, 1995; Sands *et al*, 1995; de Boer *et al*, 1999; Itoh *et al*, 2004; Yoon *et al*, 2005; Andressoo *et al*, 2006). Thus, as seen in humans, XP proteins play a major role in murine NER.

DDB1 and HR23B are two proteins involved with XPC in the recognition of DNA damage and the initiation of GG-NER. However, in contrast to $Xpc^{-/-}$ mice (Sands *et al*, 1995), *Ddb1* mutants die during early embryonic development (Cang *et al*, 2006). Inactivation of Ddb1 in developing CNS and lens resulted in massive p53-dependent apoptosis of dividing cells and lethality just after birth (Cang *et al*, 2006). MEFs deficient in Ddb1 showed defective proliferation and were UV-sensitive. A total of 90% of *HR23B* mutants suffer intrauterine or neonatal death (Ng *et al*, 2002). The surviving $HR23B^{-/-}$ mice were growth retarded and males were sterile; however, their NER and UV sensitivity remained normal. In contrast, mutants for *HR23A*, another homologue of the *S. cerevisiae* NER gene *Rad23*, are viable and their cells proficient in UV responses (Ng *et al*, 2003). However, mutations of both *HR23A* and *HR23B* lead to embryonic lethality and increased UV sensitivity, showing a redundancy between these two NER proteins (Ng *et al*, 2003). It remains to be shown whether inactivation of *Ddb1* or *mHR23A/B* would facilitate spontaneous or UV-induced cancer in mouse models.

Table III Examples of mouse models for the NER pathway

Genotype	Developmental defects	Fertility defects	Induced tumorigenesis	References
Xpd−/−	Pre-implantation embryonic lethality	NA	NA	(de Boer *et al*, 1998b)
$Xpd^{R722W/R722W}$	Growth retardation	None	UV- and DMBA-induced skin cancer	(de Boer *et al*, 1998a, 1999)
$Xpd^{G602D/G602D}$	Growth retardation	None	Early onset of UV-induced skin and/or eye tumours	(Andressoo *et al*, 2006)
Xpe−/−	None	None	UV-induced skin cancer	(Itoh *et al*, 2004; Yoon *et al*, 2005)
Xpa−/−	None	None	UV- and DMBA-induced skin cancer	(Nakane *et al*, 1995)
Xpc−/−	None	None	UV-induced skin cancer	(Sands *et al*, 1995)
Xpg−/−	Growth retardation and premature death	NA	NA	(Harada *et al*, 1999)
Ddb1−/−	Early embryonic lethality	NA	NA	(Cang *et al*, 2006)
HR23A−/−	Unaffected	None	NT	(Ng *et al*, 2002)
HR23B−/−	Intrauterine/neonatal death. 10% viable but growth retarded	Male infertility	NT	(Ng *et al*, 2002)
HR23A−/−; *HR23B−/−*	Embryonic lethality	NA	NA	(Ng *et al*, 2003)
Csa−/−	None	None	UV-induced skin cancer	(van der Horst *et al*, 1997)
Csb−/−	None	None	UV-induced skin cancer	(van der Horst *et al*, 2002)
Ercc1−/−	Growth retardation and death before weaning	NA	NA	(McWhir *et al*, 1993; Weeda *et al*, 1997)
Xpf−/−	Growth retardation and death before weaning	NA	NA	(Tian *et al*, 2004b)

NA, not applicable; NT, not tested.

Mice mutant for *Csa* (*Ercc8*) or *Csb* (*Ercc6*) have been generated (van der Horst *et al*, 1997, 2002). These mutants are viable, but their cells, although competent in GG-NER, are nonetheless impaired in TC-NER and are UV-sensitive. Surprisingly, in contrast to CS individuals, *Csa*- and *Csb*-mutant mice are prone to UV-induced skin cancer. This discrepancy is also evident in TTD mouse models, as in contrast to TTD individuals, Xpd^{R722W} mice are susceptible to UV-induced skin cancer (de Boer *et al*, 1999).

As mentioned earlier, cross talk exists between DNA-damage repair pathways and certain DNA-damage repair proteins. For example, during NER, the endonuclease ERCC1/XPF cleaves a DNA strand on the 5′-side of the lesion, allowing the repair to proceed. However, ERCC1/XPF is also implicated in interstrand crosslink (ICL) repair and HR, suggesting that the phenotypes observed in *Ercc1* or *Xpf* mutants are unlikely to result only from the impairment of NER. Cells deficient in Ercc1 or Xpf show high sensitivity to UV and to the ICL mitomycin C (MMC), and mice with inactivation of *Ercc1* or *Xpf* are runted, dying at approximately 3–4 weeks of age (McWhir *et al*, 1993; Weeda *et al*, 1997; Tian *et al*, 2004b).

Thus, NER is an important DNA-repair pathway and its impairment is associated with growth defects, excessive UV sensitivity, and in certain cases, increased skin cancer.

The BER pathway

The BER pathway deals with base damage, the most common insult to cellular DNA (Figure 1; Wilson and Bohr, 2007). Two sub-pathways, short-patch BER and long-patch BER, are involved in BER. The short-patch BER sub-pathway typically replaces a single nucleotide, whereas the long-patch sub-pathway results in the incorporation of 2–13 nucleotides. The two sub-pathways progress through different major processes that initially involve the removal of the damaged base by glycosylases such as Ogg1 and Mutyh (Myh). This is followed by strand incision of the apurinic or apurimidinic (AP) site by the endonuclease APE1. The newly generated gap is filled by incorporation of nucleotide(s), mediated by DNA polymerase-β (Polβ) in the case of the short-patch BER sub-pathway, and by Polβ and/or Polε or δ in the case of the long-patch BER sub-pathway. Strand ligation is carried out by the XRCC1/ligase III (LigIII) complex in the case of the short-patch sub-pathway. For the long-patch BER sub-pathway, other proteins are involved in the repair process before the ligation takes place. Thus, FEN1, PARP1, and LigI participate in the DNA synthesis–ligation step and displace the 2- to 13-base DNA flap. FEN1, a 5′-flap endonuclease and 5′–3′ exonuclease, excises the flap and the strand ligation is carried out by LigI.

For a period of time, little evidence existed to support the involvement of BER in human cancer or any other disorders. However, recent studies have demonstrated the existence of a human disorder linked to defective BER (Figure 2). This autosomal recessive disorder, referred to as MUTYH-associated polyposis (MAP), is associated with biallelic germline mutations of the human *MUTYH*, and is characterized by multiple colorectal adenomas and carcinomas (Cheadle and Sampson, 2007). The glycosylase MUTYH, a bacterial *mutY* homologue, functions in the BER of oxidative DNA damage by excising adenines misincorporated opposite 8-oxoG. Deficient repair of this damage results in G:C→T:A mutations, typically found in the adenomatous polyposis coli (*APC*) gene in MAP tumors.

In addition to Myh, several mammalian glycosylases are involved in BER, including Nth1, Ogg1, Ung, and Aag.

Murine homozygous mutants for the glycosylases *Nth1*, *Ogg1*, *Ung*, *Aag*, and *Mutyh* have been generated by gene targeting, and surprisingly, these mutants were viable (Table IV). *Nth1*$^{-/-}$, *Ogg1*$^{-/-}$, and *Aag*$^{-/-}$ mice showed no overt abnormalities (Engelward *et al*, 1997; Klungland *et al*, 1999; Ocampo *et al*, 2002; Takao *et al*, 2002); however after a long latency, *Ung*$^{-/-}$ and *Mutyh*$^{-/-}$ mice developed B-cell lymphomas and intestinal tumors, respectively (Nilsen *et al*, 2003; Sakamoto *et al*, 2007). These findings suggest functional redundancy of certain BER glycosylases. This is further supported by the pronounced phenotypes of *Ogg1*$^{-/-}$ *Mutyh*$^{-/-}$ mice; these mice had shorter life span and elevated risk for cancer, with 50% of double mutants developing lung tumors, lymphomas, sarcomas, and others tumors by 15 months of age (Xie *et al*, 2004).

In contrast to glycosylases, targeted inactivation of enzymes that act downstream of the glycosylases in BER resulted in embryonic or post-natal lethality. Thus, homozygous mutants for *Ape1* (Ludwig *et al*, 1998), *LigI* (Petrini *et al*, 1995), *LigIII* (Puebla-Osorio *et al*, 2006), *Xrcc1* (Tebbs *et al*, 1999), or *Flap endonuclease 1* (*Fen1*) (Kucherlapati *et al*, 2002) died during embryonic development, whereas homozygous mutants for *Pol*β died immediately after birth (Gu *et al*, 1994; Sugo *et al*, 2000). The death of mutants such as *Xrcc1*$^{-/-}$, *LigIII*$^{-/-}$ and *Pol*β$^{-/-}$ was preceded by elevated levels of apoptosis (Gu *et al*, 1994; Tebbs *et al*, 1999; Sugo *et al*, 2000; Puebla-Osorio *et al*, 2006). Interestingly, inactivation of p53 rescued the apoptosis but not the lethality of these mutants, suggesting the contribution of other p53-independent mechanisms in the deaths of these mutants.

Despite the presumed important role for the BER pathway in maintaining genomic integrity, mutations in this pathway have not significantly predisposed mutant mice for cancer. This is in contrast to mutations of other excision repair pathways, such as NER and MMR.

The HR repair pathway

DSB repair can be mediated by two major repair pathways depending on the context of the DNA damage, HR or NHEJ repair pathways (Figure 1; Kanaar *et al*, 2008). In bacteria and yeast, DSBs are preferentially repaired by HR, whereas more than 90% of DSB in mammalian cells are repaired by NHEJ. Both pathways are well defined and their impairment is associated with defects and pathologies, including increased cell death, cell-cycle arrest, telomere defects, genomic instability, meiotic defects, immunodeficiency, and cancer (Krogh and Symington, 2004; Sung and Klein, 2006).

HR is a multistep process that requires several proteins and operates at the S or G2 phase of the cell cycle. Although it accounts only for the repair of ~10% of DSBs in mammalian cells, HR defects can have severe consequences, as demonstrated by the human syndromes AT-like disorder (ATLD) and the NBS (Figure 2; Thompson and Schild, 2002). The predisposition to either syndrome has been linked to mutations in the MRN complex, which is important for the resection of DSBs (Thoms *et al*, 2007). However, it is important to note MRN functions are not restricted to HR, as is also involved in NHEJ, checkpoint activation, and telomere maintenance (Niida and Nakanishi, 2006).

The very rare human ATLD is associated with hypomorphic mutations in the *MRE11* gene (Taylor *et al*, 2004). Patients with this disorder exhibit clinical features similar to AT, including immunodeficiency and progressive neurological degeneration (Thoms *et al*, 2007). However, in contrast to AT, ATLD is not associated with ocular telangiectasia and the course of the disease is considerably milder. Similar to AT and NBS, cellular features of ATLD include defective DSB repair and repair-related cell responses, hypersensitivity to IR, as well as increased spontaneous and IR-induced genomic instability.

The NBS is a rare human autosomal recessive disorder caused by hypomorphic *NBS1* (*NIBRIN*) mutations (Thoms *et al*, 2007). This disorder is characterized by growth retardation, immunodeficiency, microcephaly, and cancer predispositions, particularly lymphomas. Cellular characteristics of NBS include radiosensitivity, increased levels of spontaneous and IR-induced chromosome breakage, and defective cell-cycle checkpoints.

Table IV Examples of mouse models for the BER pathway

Genotype	Developmental defects	Fertility defects	Spontaneous tumorigenesis	References
Nth1−/−	None	None	Not affected	(Ocampo *et al*, 2002; Takao *et al*, 2002)
Ogg1−/−	None	None	Not affected	(Klungland *et al*, 1999)
Ung−/−	None	None	Late onset of B-cell lymphomas	(Nilsen *et al*, 2003)
Aag−/−	None	None	Not affected	(Engelward *et al*, 1997)
Mutyh−/−	None	None	Late onset of intestinal tumours	(Sakamoto *et al*, 2007)
Ogg1−/− *Mutyh−/−*	None	None	Late onset of lung tumours, lymphomas, sarcomas, and others tumours	(Xie *et al*, 2004)
Ape1−/−	Embryonic lethality	NA	NA	(Ludwig *et al*, 1998)
LigI−/−	Embryonic lethality	NA	NA	(Petrini *et al*, 1995)
LigIII−/−	Embryonic lethality	NA	NA	(Puebla-Osorio *et al*, 2006)
Xrcc1−/−	Embryonic lethality	NA	NA	(Tebbs *et al*, 1999)
Fen1−/−	Embryonic lethality	NA	NA	(Kucherlapati *et al*, 2002)
*Pol*β*−/−*	Death immediately after birth	NA	NA	(Gu *et al*, 1994; Sugo *et al*, 2000)

NA, not applicable.

In contrast to both *MRE11* and *NBS1*, no data have yet been reported to support a role for *RAD50* in human chromosomal breakage or immunodeficiency syndromes.

The functions of the MRN complex have been studied in various organisms. *S. cerevisiae* strains carrying null mutations in components of the Mre11p–Rad50p–Xrs2p (MRX) complex have been generated, where Xrs2p is the functional homologue of mammalian NBS1. MRX mutants are viable, hypersensitive to IR and MMS, and are defective in meiotic recombination (Krogh and Symington, 2004). Mutations of the MRN complex have also been assessed in DT40. This chicken B-lymphocyte cell line is deficient in p53, but highly competent for homologous recombination and gene targeting. Deficiency of Mre11 in DT40 is lethal and its conditional inactivation leads to radiosensitivity, defective proliferation, genomic instability, and decreased HR (Yamaguchi-Iwai *et al*, 1999). DT40 deficient in NBS1 also display increased IR sensitivity, abnormal S-phase checkpoints, and decreased HR (Tauchi *et al*, 2002).

In contrast to *S. cerevisiae*, null mutations of *Mre11* (Xiao and Weaver, 1997), *Nbs1* (Zhu *et al*, 2001), and *Rad50* (Luo *et al*, 1999) result in early mouse embryonic lethality (Table V). Therefore, to circumvent embryonic lethality, hypomorphic and tissue-specific mutants for the three MRN components were generated. $Mre11^{ATLD1/ATLD1}$-mutant mice carrying an A to T substitution at amino acid 1894 of Mre11, which results in a 75-amino-acid truncation, exhibited reduced female fertility, defective ATM functions, and increased genomic instability. However, no cancer phenotypes were observed (Theunissen *et al*, 2003).

$Rad50^{s}$ ($Rad50^{k22M}$) hypomorphic mutant mice have also been generated (Bender *et al*, 2002). Approximately 40% of $Rad50^{s/s}$ mutants die *in utero*; however, those that are viable show progressive haematopoietic failure, short life span, and increased predisposition to thymic lymphoma. In contrast to $rad50^{s}$ in yeast, $Rad50^{s/s}$ did not impair meiotic progression and the mutants were fertile; however, p53-mediated apoptosis was increased in the testes. Inactivation of the

Table V Examples of mouse models for the HR pathway

Genotype	Developmental defects	Fertility defects	Spontaneous tumorigenesis	References
Mre11−/−	Early embryonic lethality	NA	NA	(Xiao and Weaver, 1997)
$Mre11^{ATLD1/ATLD1}$	None	Reduced female fertility	None	(Theunissen *et al*, 2003)
Rad50−/−	Early embryonic lethality	NA	NA	(Luo *et al*, 1999)
$Rad50^{s/s}$ ($Rad50^{k22M/k22M}$)	40% die *in utero*. The one that survive show haematopoietic failure	None	Thymic lymphoma	(Bender *et al*, 2002)
Nbs1−/−	Early embryonic lethality	NA	Mild cancer predisposition of *Nbs1+/−* mice	(Zhu *et al*, 2001)
$Nbs1^{\Delta 2-3/\Delta 2-3}$	Growth retardation, lymphoid defects	Female sterility	Thymic lymphomas	(Kang *et al*, 2002)
$Nbs1^{\Delta 4-5/\Delta 4-5}$	None	None	Twofold increase	(Williams *et al*, 2002)
Rad52−/−	None	None	Not affected	(Rijkers *et al*, 1998)
Rad51−/−, *Rad51B−/−* *Rad51D−/−*	Embryonic lethality	NA	NA	(Lim and Hasty, 1996; Tsuzuki *et al*, 1996a; Shu *et al*, 1999; Pittman and Schimenti, 2000)
Xrcc2−/−	Embryonic lethality	NA	NA	(Deans *et al*, 2003; Orii *et al*, 2006; Adam *et al*, 2007)
Dmc1−/−	None	Mutants are sterile	Not affected	(Pittman *et al*, 1998; Yoshida *et al*, 1998)
Rad54−/−, *Rad54B−/−*, and *Rad54−/−*; *Rad54B−/−*	None	None	Not affected	(Essers *et al*, 1997; Bross *et al*, 2003; Wesoly *et al*, 2006)
Brca1−/−	Embryonic lethality	NA	NA	(Gowen *et al*, 1996; Hakem *et al*, 1996; Liu *et al*, 1996; Ludwig *et al*, 1997; Xu *et al*, 2001)
Brca1 mutation in mammary epithelial cells		NA	Mammary tumorigenesis that is enhanced on *Chk2*- or *p53*-mutant backgrounds	(Xu *et al*, 1999; McPherson *et al*, 2004b)
Brca2−/−	Early embryonic lethality	NA	NA	(Ludwig *et al*, 1997; Suzuki *et al*, 1997)
Brca2 mutation in mammary epithelial cells			Mammary tumorigenesis on p53-mutant background	(Jonkers *et al*, 2001)
Blm−/−	None	None	Predisposition to a wide range of tumours	(Luo *et al*, 2000)
Mus81−/−	None	None	T- and B-cell lymphomas	(McPherson *et al*, 2004a)
Mus81−/−p53−/−	Female embryonic lethality	None	Multiples tumours including lymphomas and sarcomas	(Pamidi *et al*, 2007)

NA, not applicable.

Atm–Chk2–p53 pathway by the hypomorphic mutation *Mre11*^{ATLD1} or the *Nbs1*$^{\Delta B}$ (*Nbs1*$^{\Delta 4-5}$) mutation, was able to rescue the depletion of haematopoeitic cells in the *Rad50*$^{s/s}$ mutants (Morales *et al*, 2005). Surprisingly, tumorigenesis, senescence, and radiosensitivity associated with *Atm* mutation were all partially suppressed by the *Rad50*^{s} mutation (Morales *et al*, 2005). Although the exact mechanism for this rescue is not fully understood, it might involve compensatory activation of other checkpoint pathways, such as the ATR pathway.

Similar to *Rad50* and *Mre11* mutations, *Nbs1*-null mutations resulted in early embryonic lethality (Zhu *et al*, 2001; Dumon-Jones *et al*, 2003). In contrast, *Nbs1*$^{\Delta 2-3}$*and* *Nbs1*$^{\Delta 4-5}$ hypomorphic mutants were viable (Kang *et al*, 2002; Williams *et al*, 2002). Cells from these mutants were defective in the intra-S-phase and G2/M checkpoints. Heterozygous mice carrying a null *Nbs1* mutation (Dumon-Jones *et al*, 2003), as well as homozygous mice carrying *Nbs1*$^{\Delta 2-3}$*or* *Nbs1*$^{\Delta 4-5}$ hypomorphic mutations (Kang *et al*, 2002; Williams *et al*, 2002), demonstrated a mild predisposition for cancer. Interestingly, whereas p53 inactivation shortened the tumor latency of *Nbs1*$^{\Delta 4-5}$ mutants, Atm inactivation, or its impaired function on *Mre11*$^{ATLD1/ATLD1}$-mutant background, resulted in synthetic embryonic lethality of *Nbs1*$^{\Delta 4-5}$ mutants (Williams *et al*, 2002; Morales *et al*, 2005).

In addition to the previous models, a conditional mutant strain for *Nbs1* has also been generated (Frappart *et al*, 2005). Neuronal inactivation of *Nbs1* in these mice leads to chromosomal breaks, microcephaly, growth retardation, cerebellar defects, and ataxia, representing a combination of features characteristic of NBS, AT, and ATLD. p53 inactivation in this model also significantly rescued the neurological defects associated with *Nbs1* mutations (Frappart *et al*, 2005).

Thus, the MRN complex is essential for maintaining genomic integrity, cell viability, and checkpoint activation. Moreso, its requirement in various species demonstrates its essential conserved functions.

Similar to Rad50, Rad52 is a member of the Rad52 epistasis group of proteins originally identified by their requirement for the repair of IR-induced DNA damage (Krogh and Symington, 2004). Inactivation of *rad52* in *S. cerevisiae* results in increased radiation sensitivity and decreased recombination (Symington, 2002). However, its inactivation in DT40 reduced gene-targeting frequency, but did not lead to increased IR sensitivity and viability and growth of the mutant cells were not affected (Yamaguchi-Iwai *et al*, 1998). The effects of inactivation of mouse Rad52 were also investigated. Null *Rad52* mouse embryonic stem (ES) cells obtained by gene targeting demonstrated a 30–40% decrease in the frequency of HR compared with controls (Rijkers *et al*, 1998). In contrast to *rad52* mutants in *S. cerevisiae*, Rad52-deficient ES cells were not hypersensitive to IR or agents that induce DSBs. *Rad52*$^{-/-}$ mice, were viable, fertile, and showed no overt abnormalities (Rijkers *et al*, 1998). *Rad52* inactivation was shown to extend the life span of *Atm*$^{-/-}$ mice, partially rescue their T-cell development, and suppress/delay their tumorigenesis (Treuner *et al*, 2004). However, growth defects, infertility, and radiosensitivity of *Atm*$^{-/-}$ mice were not rescued on by a *Rad52*-mutant background. The reasons for the rescue of certain *Atm*$^{-/-}$ phenotypes by Rad52 inactivation are not clear, but nevertheless this is reminiscent of the rescue mediated by *Rad50*$^{s/s}$ mutation of tumorigenesis, senescence, and radiosensitivity associated with Atm inactivation (Morales *et al*, 2005).

The mammalian Rad51 family is also important for HR. This family is composed of RAD51, disrupted meiotic cDNA 1 (Dmc1), and five RAD51 paralogues, RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3. RAD51, a homologue of the bacterial DNA-dependent ATPase, RecA, is central for the homology search and strand exchange during HR.

Mutants *rad51* in *S. cerevisiae* are viable, IR sensitive, have meiotic defects, and accumulate meiosis-specific double-strand breaks (Shinohara *et al*, 1992). Rad51 deficiency in DT40 leads to chromosomal breakage, G2/M arrest, and cell death (Sonoda *et al*, 1998). *Rad51*-null mutation in mice results in early embryonic lethality associated with chromosomal loss, radiosensitivity, decreased cell proliferation, and increased apoptosis (Lim and Hasty, 1996; Tsuzuki *et al*, 1996a).

Mutants for *Rad51* paralogues were also generated. Thus, for example, inactivation of Rad51 paralogues in DT40 did not compromise cell survival (Takata *et al*, 2000, 2001). However, decreased gene-targeting efficiency, increased spontaneous, and induced cell death in response to IR or MMC, as well as elevated chromosomal aberrations, were observed in these mutants. Mutant mice for the *Rad51* paralogue *Rad51B* (Shu *et al*, 1999), *Rad51D* (Pittman and Schimenti, 2000), or *Xrcc2* (Deans *et al*, 2003) were also generated. These mutations resulted in lethality at different stages of embryonic development and their effects on cell growth were variable. Consistent with the role of Rad51 and its paralogues in HR, their mutations result in accumulation of DNA damage and activation of cellular checkpoints, including p53. Consequently, the embryonic lethality of *Rad51* (Lim and Hasty, 1996), *Rad51 B* (Shu *et al*, 1999), and *Rad51 D* (Smiraldo *et al*, 2005) mutants was delayed on *p53*-null background. The role of p53 in the embryonic lethality of *Xrcc2*$^{-/-}$ mutants remains controversial (Orii *et al*, 2006; Adam *et al*, 2007).

The meiosis specific the Rad51 paralogue Dmc1 is essential for meiotic recombination in *S. cerevisiae* (Bishop *et al*, 1992). In mice, Dmc1 expression is restricted to the testis and ovaries, and null *Dmc1* mutants are sterile and exhibit arrested gametogenesis in the first meiotic prophase (Pittman *et al*, 1998; Yoshida *et al*, 1998). These results demonstrate the conservation of the essential meiotic function of Dmc1.

Rad54 is another member of the Rad52 epistasis group involved in HR. Similar to *rad51* and *rad52* mutants, *S. cerevisiae* deficient in rad54 are highly sensitive to IR. DT40 cells mutant for *Rad54* are viable, hypersensitive to IR, exhibit slow growth, decreased rate of Ig gene conversion, and show a drastic decrease in gene-targeting efficiency (Bezzubova *et al*, 1997). Rad54 paralogues exist and include rdh54 in *S. cerevisiae* and Rad54B in mammalian cells. In contrast to *rad54* mutants in *S. cerevisiae*, *rdh54* mutants are not sensitive to IR and show no defects in mitosis (Shinohara *et al*, 1997). However, meiotic recombination was affected in *rdh54* mutants and was completely defective in *S. cerevisiae* lacking the two Rad54 orthologues (Shinohara *et al*, 1997).

Homozygous mutant mice lacking Rad54 and/or its paralogue Rad54B are viable and ES cells deficient in Rad54 or Rad54B are hypersensitive to IR, MMS, and MMC (Essers

et al, 1997; Bross *et al*, 2003; Wesoly *et al*, 2006). *Rad54*$^{-/-}$, but not *Rad54B*$^{-/-}$, ES cells have a 3- to 40-fold decrease in HR as assessed by gene targeting. Dual inactivation of both paralogues further impairs HR, as double mutant ES cells show a further 10-fold reduction in their gene targeting efficiency compared with *Rad54*-null ES cells. Although Rad54 is important for HR, mice deficient in Rad54 and/or Rad54B do not have a predisposition to cancer.

BRCA1 and *BRCA2*, the early-onset breast cancer-susceptibility genes, have also been demonstrated to partake in HR-mediated DSB repair. Germline mutations of *BRCA1* or *BRCA2* predispose women to familial human breast and ovarian cancer (Narod and Foulkes, 2004). Individuals with germline mutations for *BRCA1* or *BRCA2* also have increased risk for other cancers, including prostate cancer. Although the *BRCA1* gene has important functions in DNA-damage signalling/repair it is also involved in other cellular processes, including transcription, ubiquitination, oestrogen receptor signalling, and chromatin remodelling. In response to DSBs, BRCA1 is phosphorylated by ATM, ATR, and Chk2, underscoring its functional regulation by other molecules involved in DNA-damage checkpoints. BRCA2 functions in the loading of the HR protein RAD51 during filament formation. It directly binds to RAD51 and its phosphorylation on Ser3291 inhibits this binding (Esashi *et al*, 2005). In addition to its role in HR, recent studies have suggested a role for BRCA2 in the stabilization of stalled RFs (Lomonosov *et al*, 2003).

Homozygous mutations of murine *Brca1* resulted in embryonic lethality. (Gowen *et al*, 1996; Hakem *et al*, 1996; Liu *et al*, 1996; Ludwig *et al*, 1997; Xu *et al*, 2001). Premature death of *Brca1*-mutant embryos is likely due to accumulation of damaged DNA and activation of DNA-damage checkpoints, including Chk2–p53. Inactivation of p53 delayed the embryonic lethality of *Brca1*-null mutants (Hakem *et al*, 1997; Ludwig *et al*, 1997) and completely rescued survival of *Brca1* hypomorphic mutants (Xu *et al*, 2001). Studies of hypomorphic and conditional null *Brca1* mutant females demonstrated that similar to humans, *Brca1* mutations increase the risk for mammary cancer (Xu *et al*, 1999; McPherson *et al*, 2004b). Interestingly, inactivation of p53, or Chk2, drastically facilitates development of mammary tumors on *Brca1*-mutant backgrounds (Xu *et al*, 1999; McPherson *et al*, 2004b). A targeted *Brca1*-null mutation to the T-cell lineage resulted in increased genomic instability, apoptosis, cell-cycle arrest, and a drastic depletion of the T-cell lineage (Mak *et al*, 2000). Interestingly, development of *Brca1*-null T-cells was completely rescued in *p53*- or *Chk*2-mutant backgrounds, and this rescue was at the expense of increased genomic instability and increased risk for tumorigenesis (McPherson *et al*, 2004b).

BRCA1 plays a major role in the DNA-damage response as it interacts with the MRN complex and γ-H2AX at the site of DSB. Likewise, it is also required for the recruitment of other proteins such as Rad51, BRCA2, and BARD1 to sites of DSBs (Greenberg *et al*, 2006). HR, as measured by the efficiency of gene targeting in ES cells, was drastically reduced in *Brca1* hypomorphic mutants (Moynahan *et al*, 1999). Other defects, including compromised telomeres integrity and male sterility, were also associated with *Brca1* mutations (Xu *et al*, 2001; McPherson *et al*, 2006).

Similar to *Brca1* and *Rad51* mutants, null mutations for *Brca2* result in early mouse embryonic lethality and impaired HR (Ludwig *et al*, 1997; Suzuki *et al*, 1997; Moynahan *et al*, 2001). Furthermore, loss of Brca2 in murine cells resulted in increased genomic instability and activation of p53. A small subset of *Brca2* hypomorphic mutants survived embryonic development (Connor *et al*, 1997; Friedman *et al*, 1998). These mice were growth defective, sterile, and predisposed for thymic lymphoma, and their cells were sensitive to DNA damaging agents, including IR, MMS, and UV radiation. Conditional mutant strains have also been generated using the *Cre/loxP* system and similar to *Brca1* mutation, dual inactivation of *Brca2* and *p53* in mammary epithelial cells resulted in increased frequency and decreased latency for mammary tumors (Jonkers *et al*, 2001).

Beside the proteins described above, efficient HR requires other proteins such as members of the family of RecQ helicases (Hickson, 2003). The human family of RecQ helicases includes BLM, REQ, WRN, RECQL4, and RECQL5, and is important for unwinding DNA, repairing stalled RFs, promoting HR, and maintaining overall genomic integrity. Mutations of some of these helicases are associated with rare human syndromes (Hickson, 2003). Thus, the Werner syndrome (WS) and the Rothmund Thomson syndrome (RTS) are associated with mutations of human *WRN* and *RECQL4* genes, respectively (Figure 2). WS features include premature ageing, short stature, and cancer predisposition. Clinical features of RTS include short stature, skin pigmentation changes, skin atrophy, and increased cancer predisposition.

In addition mutations of the human *BLM* gene are associated with the Bloom syndrome (BS), a rare autosomal recessive disorder characterized by growth defects, immune deficiency, reduced fertility, and predisposition to a large spectrum of cancers. Cells from BS patients have elevated sister-chromatid exchange (SCE) and genomic instability. Three mouse models for *Blm* mutation have been generated, but only one was viable (Chester *et al*, 1998; Luo *et al*, 2000; Goss *et al*, 2002). Similar to BS patients, viable *Blm* mice are prone to a wide range of tumors, and cells from these mutant mice show elevated levels of SCE, a hallmark cellular phenotype of BS. In addition, Blm deficiency leads to increased mitotic recombination and somatic loss of heterozygosity (Luo *et al*, 2000).

While most steps essential for HR in eukaryotes have been well characterized, the identification of the resolvase(s) required for the resolution of Holliday junctions (HJs) during mitotic and meiotic recombination turned out to be very challenging. Resolution of HJs is critical for HR and is mediated in *E. coli by* the resolvase *RuvC* (West, 1997); however, the search for true HJ resolvase(s) in eukaryotes is still ongoing.

Yeast Mus81 (MMS, UV-sensitive, clone 81) with its partner Eme1 (*Schizosaccharomyces pombe*) or MMS4 (*S. cerevisiae*) forms a structure-specific endonuclease important for DNA-damage repair (Osman and Whitby, 2007). Yeast *mus81*, *eme1*, or *mms4* mutants show increased sensitivity to DNA-damaging agents that interfere with normal progression of RFs caused by agents such as UV radiation, MMS, and camptothecin, but their sensitivity to IR is not affected (Osman and Whitby, 2007) . These mutants also show meiotic defects supporting a role for this endonuclease in this process.

In vitro studies indicate that yeast and mammalian Mus81, with their partner Eme1/Mms4, efficiently cleaves various

DNA structures that mimic RFs, D-loops, and nicked HJs, but the cleavage activity of intact HJs was weak (Osman and Whitby, 2007). Thus, at least in mammalian cells, Mus81 with Eme1 or with Eme2, another eme1 homologue, forms a heterodimeric 3′-flap/RF endonucleases that process stalled RFs and recombination intermediates, but does not possess typical characteristics of an HJ resolvase (Abraham *et al*, 2003; Ciccia *et al*, 2003, 2007; Ogrunc and Sancar, 2003).

Gene-targeted inactivation of *Mus81* or *Eme1* in mouse and human cells increased sensitivity to ICL, hydroxyurea, but not UV or IR and enhanced spontaneous and MMC-induced genomic instability (Abraham *et al*, 2003; McPherson *et al*, 2004a). Mus81 was shown to be important for generating ICL-induced DSBs, as well as for mediating the restart of stalled or blocked RFs (Hanada *et al*, 2006). Our studies of a mouse model for *Mus81*-null mutation demonstrated that Mus81 is a haploinsufficient tumor suppressor (McPherson *et al*, 2004a). Heterozygous and homozygous *Mus81* mutants show cancer predisposition, particularly to T- and B-cell lymphomas. The MMC hypersensitivity of *Mus81*-mutant cells and mice is p53-dependent (Pamidi *et al*, 2007). Interestingly, on a *p53*-null background, *Mus81*-mutant mice have more genomic instability and develop sarcomas and multiple different tumors with short latency and high penetrance, demonstrating a role for p53 in suppressing cancer associated with *Mus81* mutation (Pamidi *et al*, 2007). Both *Mus81*- and *Mus81p53*-mutant mice are fertile, thus failing to support a requirement for Mus81 in meiotic recombination. A second *Mus81*-mutant strain ($Mus81^{\Delta 9-12}$) showed increased genomic instability but failed to show increased tumorigenesis (Dendouga *et al*, 2005). A role in cancer for Eme1 or Eme2 requires further investigations.

Recent studies have implicated the mammalian RAD51 paralogues RAD51C–XRCC3 in HJ resolution (Liu *et al*, 2004). Rad51C–XRCC3 binds HJs *in vitro* and cell extracts from Rad51C- or XRCC3-deficient hamster cells, exhibit low HJ resolvase activities. In addition, depletion of RAD51C from HeLa cell extracts strongly impairs *in vitro* HJ branch migration and resolution. Similar to other Rad51 paralogues, null mutation of mouse *Rad51C* results in embryonic lethality. However, a viable mouse model carrying intronic integration of a *Neomycine* selection cassette resulting in decreased Rad51 expression has been recently reported (Kuznetsov *et al*, 2007). About 40% of mutant males and 10% of mutant females were infertile. Cell extracts from MEFs null for *Rad51C* and *p53* show reduced *in vitro* HJ resolvase activities compared with controls. Thus, the current data support a role for the mammalian RAD51C in HJ resolution; however, further studies are still required to establish RAD51C as a typical HJ resolvase and to demonstrate whether other mammalian HJ resolvases also exist.

Thus, the human syndromes and early onset of breast cancer, and the developmental defects, increased genomic instability and tumorigenesis associated with impaired HR in mouse models, all demonstrate the *in vivo* requirement for HR-mediated DNA-damage repair.

The NHEJ repair pathway

NHEJ is the predominant pathway of DSBs repair in mammalian cells (Figure 1; Kanaar *et al*, 2008). This repair pathway is active especially at the G1, but is error prone. NHEJ is also essential for T-cell receptor-α/β and Ig V(D)J recombination, and thus this repair pathway is required for the development of the T and B-cell repertoires. The core protein components of the mammalian NHEJ include the Ku subunits (Ku70 and Ku80), DNA–PKcs, XRCC4, DNA ligase IV (LigIV), Artemis, and the recently identified Cernunnos–XLF (also known as NHEJ1).

DNA–PK is composed of the catalytic subunit DNA–PKcs and the heterodimer Ku70/Ku80, important for DNA end binding. DNA–PKc is a serine/threonine kinase that is activated following its recruitment by Ku70/Ku80 to sites of DSBs. Active DNA–PKcs autophosphorylate themselves as

Table VI Examples of mouse models for the NHEJ repair pathway

Genotype	Developmental defects	Fertility defects	Spontaneous tumorigenesis	References
DNA-PKcs−/−	T and B-cell development arrested at early progenitor stages	None	Not affected	(Gao *et al*, 1998a; Taccioli *et al*, 1998; Kurimasa *et al*, 1999)
Ku70−/−	Growth retardation and early arrest of T and B-cell development	None	Thymic lymphomas	(Gu *et al*, 1997; Ouyang *et al*, 1997)
Ku80−/−	Growth retardation and early arrest of T and B-cell development	None	Not affected	(Nussenzweig *et al*, 1997)
Ku80−/−p53−/−	Growth retardation and block of T and B-cell development	None	Early onset of pro B-cell lymphomas	(Difilippantonio *et al*, 2000)
Artemis−/−	Developmental arrest at early T and B cell progenitor stages	None	None	(Rooney *et al*, 2002; Li *et al*, 2005)
Artemis−/− p53−/−	Developmental arrest at early T and B cell progenitor stages	None	Pro-B cell lymphomas	(Rooney *et al*, 2004)
$LigIV^{Y288C}$	Growth retardation and progressive loss of haematopoeitic stem cells	NA	None	(Nijnik *et al*, 2007)
LigIV−/−	Late embryonic lethality	NA	NA	(Barnes *et al*, 1998; Frank *et al*, 1998)
LigIV−/−p53−/−	Viable but growth retarded	None	Early onset of pro B-cell lymphomas	(Frank *et al*, 2000)
Xrcc4−/−	Late embryonic lethality	NA	NA	(Gao *et al*, 1998b)
Xrcc4−/−p53−/−	Viable but growth retarded	None	Early onset of pro B-cell lymphomas	(Gao *et al*, 2000)

NA, not applicable.

well as several other targets, including the Ku subunits, p53, H2AX, Artemis, XRCC4, and WRN (Collis *et al*, 2005).

The important role of DNA–PKcs *in vivo* became evident following the identification of its mutation in the severe combined immunodeficient 'SCID' mice (Bosma *et al*, 1983; Blunt *et al*, 1995; Kirchgessner *et al*, 1995). SCID mice exhibit impaired V(D)J recombination and have arrested T- and B-cell development at early progenitor stages. The role of *DNA–PKc* mutation in SCID phenotypes was confirmed in *DNA–PKc*-null mice obtained by gene targeting (Table VI; Gao *et al*, 1998a; Taccioli *et al*, 1998; Kurimasa *et al*, 1999). These mutants are severely immunodeficient, have impaired V(D)J coding joining but normal signal joining, and their T- and B-cell development are blocked at early progenitor stages. Moreso, MEFs deficient for DNA–PKcs are hypersensitive to IR, further supporting its requirement for DSB repair.

Similar to DNA–PKcs, targeted inactivation of *Ku70* or *Ku80* in mice results in SCID phenotypes associated with early arrest of T- and B-cell development, although the T-cell arrest in *Ku70* mice is leaky (Nussenzweig *et al*, 1996; Gu *et al*, 1997; Ouyang *et al*, 1997). However, in contrast to DNA–PKcs mice, null mutants for *Ku70* or *Ku80* showed growth retardation and their cells were impaired in both V(D)J coding and recombination signal (RS) end joining. Inactivation of either *Ku70* or *Ku80* resulted in elevated IR sensitivity but did not affect mice fertility. Taken together, these data demonstrate the essential role of DNA–PK in NHEJ and V(D)J recombination.

Increased apoptosis and proliferative arrest of pro-B cells in $Ku80^{-/-}$ mice were rescued by a *p53*-null background (Difilippantonio *et al*, 2000). Genomic instability associated with *Ku80* mutation was significantly increased in the absence of p53, and the double mutants developed pro B-cell lymphomas with 100% penetrance and died within 3 months of age (Nussenzweig *et al*, 1997; Difilippantonio *et al*, 2000). Similarly, the incidence of thymic lymphomas was increased in *Ku70*-null mice (Gu *et al*, 1997) and inactivation of p53 on $DNA–Pk^{scid}$-mutant background resulted in the rapid onset of lymphomas/leukaemia (Guidos *et al*, 1996).

The nuclease Artemis is a phosphorylation target for DNA–PK and forms a complex with DNA–PKcs. This complex is important for the hairpin-opening step of V(D)J recombination and for the 5′ and 3′ overhang terminal end processing in NHEJ. *ARTEMIS* mutation causes the severe combined immunodeficiency with sensitivity to ionizing radiation (RS-SCID), a rare human disorder (Figure 2; O'Driscoll and Jeggo, 2006). Similar to *DNA–PKc* mutants, mice deficient for Artemis are viable, have normal size, and suffer severe combined immunodeficiency associated with developmental arrest at early T- and B-cell progenitor stages (Rooney *et al*, 2002; Li *et al*, 2005). Artemis deficiency impaired coding but not RS joining. *Artemis*-mutant MEFs are radiosensitive and exhibit increased chromosomal instability. This spontaneous loss of genomic integrity is likely the basis for increased tumorigenesis associated with dual inactivation of Artemis and p53 (Rooney *et al*, 2004; Woo *et al*, 2007).

Following the terminal end-processing step of NHEJ, LigIV/XRCC4 complex serves to perform the ligation and final step of NHEJ. Hypomorphic mutations of human *LigIV* are associated with the hereditary autosomal LigIV syndrome (Figure 2; O'Driscoll and Jeggo, 2006). This syndrome is very rare, as only eight patients have been identified so far. This syndrome is characterized by SCID phenotype, growth defects, microcephaly, radiosensitivity, and leukaemia. Recently, Cernunnos–XLF, a novel NHEJ factor that interacts with the XRCC4–DNA LigIV complex, has been identified (Ahnesorg *et al*, 2006; Buck *et al*, 2006). Mutation of *Cernunnos–XLF* was found to be associated with a rare inherited human syndrome characterized by growth retardation, microcephaly, severe immunodeficiency, and radiosensitivity (Figure 2).

Null mutation of *LigIV* or *Xrcc4* in mouse models results in late embryonic lethality associated with extensive apoptosis in the embryonic central nervous system (Barnes *et al*, 1998; Frank *et al*, 1998; Gao *et al*, 1998b). V(D)J joining does not occur, lymphopoiesis is blocked, and MEFs deficient for LigIV or Xrcc4 are radiosensitive, growth defective and enter senescence prematurely. p53 inactivation rescued the extensive apoptosis in the central nervous system, the defective proliferation/senescence and the embryonic lethality associated with *LigIV* or *Xrcc4* mutation (Frank *et al*, 2000; Gao *et al*, 2000). However, p53 inactivation did not rescue defective V(D)J recombination or lymphocyte development of *LigIV*- or *Xrcc4*-null mutants. In addition, on a *p53*-null background, both *LigIV*- and *Xrcc4*-null mutants were growth-retarded and developed pro-B lymphomas with short latency. More recently, the hypomorphic mutation $LigIV^{Y288C}$ has been reported to lead to growth retardation, progressive loss of haematopoeitic stem cells, and immunodeficiency (Nijnik *et al*, 2007). In addition, mice carrying a targeted *Xrcc4* mutation to neuronal progenitors demonstrated increased genomic instability and predisposition for medulloblastomas (Yan *et al*, 2006). Although mice deficient for *Cernunnos–XLF* have not yet been reported, ES cells null for this gene are radiosensitive, show defective V(D)J coding and RS joining, and accumulate spontaneous genomic instability (Zha *et al*, 2007). These data suggest that similar to other NHEJ core components, inactivation of *Cernunnos–XLF* could potentially lead to cancer development.

The radiosensitivity, genomic instability, immunodeficiency, growth retardation, embryonic development, and cancer predisposition associated with defective NHEJ all demonstrate the major role this DNA-damage repair pathway plays *in vivo*.

Concluding remarks

Although there exist numerous proteins employed by several DNA-repair pathways in response to DNA damage, loss or partial inactivation of only one of these proteins can have extremely devastating consequences. Conversely, there are some mutated DNA-damage repair proteins that go unnoticed due to a lack of functional consequences at both the cellular and organism level. This is likely due to genetic redundancy and compensatory mechanisms of repair. The onset of various syndromes, increased cancer predisposition, immunodeficiency, and neurological defects associated with impaired DNA-damage repair, are all strong indications for the requirement of a tight regulation of these pathways. Our current knowledge of the mechanisms that regulate DNA repair has grown significantly over the past years. In addition to improving diagnosis, this overwhelming knowledge of the mechanism of DNA-damage repair and DNA-damage

checkpoint will likely contribute to a better design for both drugs and therapies for diseases, such as cancer.

Our understanding of the DNA-repair mechanisms in humans, and how defects in these processes lead to human syndromes and pathologies, has greatly benefited from studies in other organisms, including mice and yeast. Further studies in these organisms are required to better assess the effect of the amino-acid substitutions and point mutations of DNA-repair genes that associate with human syndromes and diseases. Characterization of the post-translational modifications that control the function and stability of DNA-repair proteins, such as phosphorylation and ubiquitination, is essential for future manipulation of the repair machinery to aid in better therapeutic responses.

Acknowledgements

We thank the members of the Hakem laboratory for helpful discussions, and Amanda Fenton, Anne Hakem, Jacinth Abraham, and Renato Cardoso for reviewing the paper. Research in the Hakem laboratory was supported by the Canadian Institutes of Health Research, the National Cancer Institute of Canada, the Cancer Research Society, and the Leukemia & Lymphoma Society Of Canada. We apologize to those whose work was not cited directly owing to space limitations.

References

Aas PA, Otterlei M, Falnes PO, Vagbo CB, Skorpen F, Akbari M, Sundheim O, Bjoras M, Slupphaug G, Seeberg E, Krokan HE (2003) Human and bacterial oxidative demethylases repair alkylation damage in both RNA and DNA. *Nature* **421:** 859–863

Abraham J, Lemmers B, Hande MP, Moynahan ME, Chahwan C, Ciccia A, Essers J, Hanada K, Chahwan R, Khaw AK, McPherson P, Shehabeldin A, Laister R, Arrowsmith C, Kanaar R, West SC, Jasin M, Hakem R (2003) Eme1 is involved in DNA damage processing and maintenance of genomic stability in mammalian cells. *EMBO J* **22:** 6137–6147

Adam J, Deans B, Thacker J (2007) A role for Xrcc2 in the early stages of mouse development. *DNA Repair (Amst)* **6:** 224–234

Ahnesorg P, Smith P, Jackson SP (2006) XLF interacts with the XRCC4–DNA ligase IV complex to promote DNA nonhomologous end-joining. *Cell* **124:** 301–313

Andressoo JO, Mitchell JR, de Wit J, Hoogstraten D, Volker M, Toussaint W, Speksnijder E, Beems RB, van Steeg H, Jans J, de Zeeuw CI, Jaspers NG, Raams A, Lehmann AR, Vermeulen W, Hoeijmakers JH, van der Horst GT (2006) An Xpd mouse model for the combined xeroderma pigmentosum/Cockayne syndrome exhibiting both cancer predisposition and segmental progeria. *Cancer Cell* **10:** 121–132

Baker SM, Bronner CE, Zhang L, Plug AW, Robatzek M, Warren G, Elliott EA, Yu J, Ashley T, Arnheim N, Flavell RA, Liskay RM (1995) Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. *Cell* **82:** 309–319

Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM (1996) Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nat Genet* **13:** 336–342

Bardwell PD, Woo CJ, Wei K, Li Z, Martin A, Sack SZ, Parris T, Edelmann W, Scharff MD (2004) Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. *Nat Immunol* **5:** 224–229

Barnes DE, Stamp G, Rosewell I, Denzel A, Lindahl T (1998) Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. *Curr Biol* **8:** 1395–1398

Becker K, Gregel CM, Kaina B (1997) The DNA repair protein *O*6-methylguanine–DNA methyltransferase protects against skin tumor formation induced by antineoplastic chloroethylnitrosourea. *Cancer Res* **57:** 3335–3338

Bender CF, Sikes ML, Sullivan R, Huye LE, Le Beau MM, Roth DB, Mirzoeva OK, Oltz EM, Petrini JH (2002) Cancer predisposition and hematopoietic failure in Rad50(S/S) mice. *Genes Dev* **16:** 2237–2251

Bezzubova O, Silbergleit A, Yamaguchi-Iwai Y, Takeda S, Buerstedde JM (1997) Reduced X-ray resistance and homologous recombination frequencies in a RAD54−/− mutant of the chicken DT40 cell line. *Cell* **89:** 185–193

Bishop DK, Park D, Xu L, Kleckner N (1992) DMC1: a meiosis-specific yeast homolog of *E. coli* recA required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell* **69:** 439–456

Blunt T, Finnie NJ, Taccioli GE, Smith GC, Demengeot J, Gottlieb TM, Mizuta R, Varghese AJ, Alt FW, Jeggo PA, Jackson SP (1995) Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation. *Cell* **80:** 813–823

Bosma GC, Custer RP, Bosma MJ (1983) A severe combined immunodeficiency mutation in the mouse. *Nature* **301:** 527–530

Bross L, Wesoly J, Buerstedde JM, Kanaar R, Jacobs H (2003) Somatic hypermutation does not require Rad54 and Rad54B-mediated homologous recombination. *Eur J Immunol* **33:** 352–357

Buck D, Malivert L, de Chasseval R, Barraud A, Fondaneche MC, Sanal O, Plebani A, Stephan JL, Hufnagel M, le Deist F, Fischer A, Durandy A, de Villartay JP, Revy P (2006) Cernunnos, a novel nonhomologous end-joining factor, is mutated in human immunodeficiency with microcephaly. *Cell* **124:** 287–299

Cang Y, Zhang J, Nicholas SA, Bastien J, Li B, Zhou P, Goff SP (2006) Deletion of DDB1 in mouse brain and lens leads to p53-dependent elimination of proliferating cells. *Cell* **127:** 929–940

Cheadle JP, Sampson JR (2007) MUTYH-associated polyposis—from defect in base excision repair to clinical genetic testing. *DNA Repair (Amst)* **6:** 274–279

Chen PC, Dudley S, Hagen W, Dizon D, Paxton L, Reichow D, Yoon SR, Yang K, Arnheim N, Liskay RM, Lipkin SM (2005) Contributions by MutL homologues Mlh3 and Pms2 to DNA mismatch repair and tumor suppression in the mouse. *Cancer Res* **65:** 8662–8670

Chester N, Kuo F, Kozak C, O'Hara CD, Leder P (1998) Stage-specific apoptosis, developmental delay, and embryonic lethality in mice homozygous for a targeted disruption in the murine Bloom's syndrome gene. *Genes Dev* **12:** 3382–3393

Chipuk JE, Green DR (2006) Dissecting p53-dependent apoptosis. *Cell Death Differ* **13:** 994–1002

Ciccia A, Constantinou A, West SC (2003) Identification and characterization of the human mus81–eme1 endonuclease. *J Biol Chem* **278:** 25172–25178

Ciccia A, Ling C, Coulthard R, Yan Z, Xue Y, Meetei AR, Laghmani el H, Joenje H, McDonald N, de Winter JP, Wang W, West SC (2007) Identification of FAAP24, a Fanconi anemia core complex protein that interacts with FANCM. *Mol Cell* **25:** 331–343

Collado M, Blasco MA, Serrano M (2007) Cellular senescence in cancer and aging. *Cell* **130:** 223–233

Collis SJ, DeWeese TL, Jeggo PA, Parker AR (2005) The life and death of DNA–PK. *Oncogene* **24:** 949–961

Connor F, Bertwistle D, Mee PJ, Ross GM, Swift S, Grigorieva E, Tybulewicz VL, Ashworth A (1997) Tumorigenesis and a DNA repair defect in mice with a truncating Brca2 mutation. *Nat Genet* **17:** 423–430

de Boer J, de Wit J, van Steeg H, Berg RJ, Morreau H, Visser P, Lehmann AR, Duran M, Hoeijmakers JH, Weeda G (1998a) A mouse model for the basal transcription/DNA repair syndrome trichothiodystrophy. *Mol Cell* **1:** 981–990

de Boer J, Donker I, de Wit J, Hoeijmakers JH, Weeda G (1998b) Disruption of the mouse xeroderma pigmentosum group D DNA repair/basal transcription gene results in preimplantation lethality. *Cancer Res* **58:** 89–94

de Boer J, van Steeg H, Berg RJ, Garssen J, de Wit J, van Oostrum CT, Beems RB, van der Horst GT, van Kreijl CF, de Gruijl FR, Bootsma D, Hoeijmakers JH, Weeda G (1999) Mouse model for

the DNA repair/basal transcription disorder trichothiodystrophy reveals cancer predisposition. *Cancer Res* **59:** 3489–3494

de Vries SS, Baart EB, Dekker M, Siezen A, de Rooij DG, de Boer P, te Riele H (1999) Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. *Genes Dev* **13:** 523–531

de Wind N, Dekker M, Berns A, Radman M, te Riele H (1995) Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* **82:** 321–330

Deans B, Griffin CS, O'Regan P, Jasin M, Thacker J (2003) Homologous recombination deficiency leads to profound genetic instability in cells derived from Xrcc2-knockout mice. *Cancer Res* **63:** 8181–8187

Dendouga N, Gao H, Moechars D, Janicot M, Vialard J, McGowan CH (2005) Disruption of murine Mus81 increases genomic instability and DNA damage sensitivity but does not promote tumorigenesis. *Mol Cell Biol* **25:** 7569–7579

Difilippantonio MJ, Zhu J, Chen HT, Meffre E, Nussenzweig MC, Max EE, Ried T, Nussenzweig A (2000) DNA repair protein Ku80 suppresses chromosomal aberrations and malignant transformation. *Nature* **404:** 510–514

Dumenco LL, Allay E, Norton K, Gerson SL (1993) The prevention of thymic lymphomas in transgenic mice by human *O*6-alkylguanine–DNA alkyltransferase. *Science* **259:** 219–222

Dumon-Jones V, Frappart PO, Tong WM, Sajithlal G, Hulla W, Schmid G, Herceg Z, Digweed M, Wang ZQ (2003) Nbn heterozygosity renders mice susceptible to tumor formation and ionizing radiation-induced tumorigenesis. *Cancer Res* **63:** 7263–7269

Duncan T, Trewick SC, Koivisto P, Bates PA, Lindahl T, Sedgwick B (2002) Reversal of DNA alkylation damage by two human dioxygenases. *Proc Natl Acad Sci USA* **99:** 16660–16665

Edelmann W, Cohen PE, Kane M, Lau K, Morrow B, Bennett S, Umar A, Kunkel T, Cattoretti G, Chaganti R, Pollard JW, Kolodner RD, Kucherlapati R (1996) Meiotic pachytene arrest in MLH1-deficient mice. *Cell* **85:** 1125–1134

Edelmann W, Cohen PE, Kneitz B, Winand N, Lia M, Heyer J, Kolodner R, Pollard JW, Kucherlapati R (1999) Mammalian MutS homologue 5 is required for chromosome pairing in meiosis. *Nat Genet* **21:** 123–127

Edelmann W, Umar A, Yang K, Heyer J, Kucherlapati M, Lia M, Kneitz B, Avdievich E, Fan K, Wong E, Crouse G, Kunkel T, Lipkin M, Kolodner RD, Kucherlapati R (2000) The DNA mismatch repair genes Msh3 and Msh6 cooperate in intestinal tumor suppression. *Cancer Res* **60:** 803–807

Edelmann W, Yang K, Umar A, Heyer J, Lau K, Fan K, Liedtke W, Cohen PE, Kane MF, Lipford JR, Yu N, Crouse GF, Pollard JW, Kunkel T, Lipkin M, Kolodner R, Kucherlapati R (1997) Mutation in the mismatch repair gene Msh6 causes cancer susceptibility. *Cell* **91:** 467–477

Engelward BP, Weeda G, Wyatt MD, Broekhof JL, de Wit J, Donker I, Allan JM, Gold B, Hoeijmakers JH, Samson LD (1997) Base excision repair deficient mice lacking the Aag alkyladenine DNA glycosylase. *Proc Natl Acad Sci USA* **94:** 13087–13092

Esashi F, Christ N, Gannon J, Liu Y, Hunt T, Jasin M, West SC (2005) CDK-dependent phosphorylation of BRCA2 as a regulatory mechanism for recombinational repair. *Nature* **434:** 598–604

Essers J, Hendriks RW, Swagemakers SM, Troelstra C, de Wit J, Bootsma D, Hoeijmakers JH, Kanaar R (1997) Disruption of mouse RAD54 reduces ionizing radiation resistance and homologous recombination. *Cell* **89:** 195–204

Falnes PO, Bjoras M, Aas PA, Sundheim O, Seeberg E (2004) Substrate specificities of bacterial and human AlkB proteins. *Nucleic Acids Res* **32:** 3456–3461

Felton KE, Gilchrist DM, Andrew SE (2007) Constitutive deficiency in DNA mismatch repair. *Clin Genet* **71:** 483–498

Frank KM, Sekiguchi JM, Seidl KJ, Swat W, Rathbun GA, Cheng HL, Davidson L, Kangaloo L, Alt FW (1998) Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. *Nature* **396:** 173–177

Frank KM, Sharpless NE, Gao Y, Sekiguchi JM, Ferguson DO, Zhu C, Manis JP, Horner J, DePinho RA, Alt FW (2000) DNA ligase IV deficiency in mice leads to defective neurogenesis and embryonic lethality via the p53 pathway. *Mol Cell* **5:** 993–1002

Frappart PO, Tong WM, Demuth I, Radovanovic I, Herceg Z, Aguzzi A, Digweed M, Wang ZQ (2005) An essential function for NBS1 in the prevention of ataxia and cerebellar defects. *Nat Med* **11:** 538–544

Friedman LS, Thistlethwaite FC, Patel KJ, Yu VP, Lee H, Venkitaraman AR, Abel KJ, Carlton MB, Hunter SM, Colledge WH, Evans MJ, Ponder BA (1998) Thymic lymphomas in mice with a truncating mutation in Brca2. *Cancer Res* **58:** 1338–1343

Gao Y, Chaudhuri J, Zhu C, Davidson L, Weaver DT, Alt FW (1998a) A targeted DNA–PKcs-null mutation reveals DNA-PK-independent functions for KU in V(D)J recombination. *Immunity* **9:** 367–376

Gao Y, Ferguson DO, Xie W, Manis JP, Sekiguchi J, Frank KM, Chaudhuri J, Horner J, DePinho RA, Alt FW (2000) Interplay of p53 and DNA-repair protein XRCC4 in tumorigenesis, genomic stability and development. *Nature* **404:** 897–900

Gao Y, Sun Y, Frank KM, Dikkes P, Fujiwara Y, Seidl KJ, Sekiguchi JM, Rathbun GA, Swat W, Wang J, Bronson RT, Malynn BA, Bryans M, Zhu C, Chaudhuri J, Davidson L, Ferrini R, Stamato T, Orkin SH, Greenberg ME *et al* (1998b) A critical role for DNA end-joining proteins in both lymphogenesis and neurogenesis. *Cell* **95:** 891–902

Glassner BJ, Weeda G, Allan JM, Broekhof JL, Carls NH, Donker I, Engelward BP, Hampson RJ, Hersmus R, Hickman MJ, Roth RB, Warren HB, Wu MM, Hoeijmakers JH, Samson LD (1999) DNA repair methyltransferase (Mgmt) knockout mice are sensitive to the lethal effects of chemotherapeutic alkylating agents. *Mutagenesis* **14:** 339–347

Goss KH, Risinger MA, Kordich JJ, Sanz MM, Straughen JE, Slovek LE, Capobianco AJ, German J, Boivin GP, Groden J (2002) Enhanced tumor formation in mice heterozygous for Blm mutation. *Science* **297:** 2051–2053

Gowen LC, Johnson BL, Latour AM, Sulik KK, Koller BH (1996) Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat Genet* **12:** 191–194

Greenberg RA, Sobhian B, Pathania S, Cantor SB, Nakatani Y, Livingston DM (2006) Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. *Genes Dev* **20:** 34–46

Gu H, Marth JD, Orban PC, Mossmann H, Rajewsky K (1994) Deletion of a DNA polymerase beta gene segment in T cells using cell type-specific gene targeting. *Science* **265:** 103–106

Gu Y, Seidl KJ, Rathbun GA, Zhu C, Manis JP, van der Stoep N, Davidson L, Cheng HL, Sekiguchi JM, Frank K, Stanhope-Baker P, Schlissel MS, Roth DB, Alt FW (1997) Growth retardation and leaky SCID phenotype of Ku70-deficient mice. *Immunity* **7:** 653–665

Guidos CJ, Williams CJ, Grandal I, Knowles G, Huang MT, Danska JS (1996) V(D)J recombination activates a p53-dependent DNA damage checkpoint in scid lymphocyte precursors. *Genes Dev* **10:** 2038–2054

Hakem R, de la Pompa JL, Elia A, Potter J, Mak TW (1997) Partial rescue of Brca1 (5–6) early embryonic lethality by p53 or p21 null mutation. *Nat Genet* **16:** 298–302

Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, Potter J, Reitmair A, Billia F, Firpo E, Hui CC, Roberts J, Rossant J, Mak TW (1996) The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell* **85:** 1009–1023

Hanada K, Budzowska M, Modesti M, Maas A, Wyman C, Essers J, Kanaar R (2006) The structure-specific endonuclease Mus81–Eme1 promotes conversion of interstrand DNA crosslinks into double-strands breaks. *EMBO J* **25:** 4921–4932

Harada YN, Shiomi N, Koike M, Ikawa M, Okabe M, Hirota S, Kitamura Y, Kitagawa M, Matsunaga T, Nikaido O, Shiomi T (1999) Postnatal growth failure, short life span, and early onset of cellular senescence and subsequent immortalization in mice lacking the xeroderma pigmentosum group G gene. *Mol Cell Biol* **19:** 2366–2372

Hickson ID (2003) RecQ helicases: caretakers of the genome. *Nat Rev Cancer* **3:** 169–178

Itoh T, Cado D, Kamide R, Linn S (2004) DDB2 gene disruption leads to skin tumors and resistance to apoptosis after exposure to ultraviolet light but not a chemical carcinogen. *Proc Natl Acad Sci USA* **101:** 2052–2057

Iwakuma T, Sakumi K, Nakatsuru Y, Kawate H, Igarashi H, Shiraishi A, Tsuzuki T, Ishikawa T, Sekiguchi M (1997) High incidence of nitrosamine-induced tumorigenesis in mice lacking DNA repair methyltransferase. *Carcinogenesis* **18:** 1631–1635

Jiricny J (2006) The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* **7:** 335–346

Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A (2001) Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. *Nat Genet* **29:** 418–425

Kanaar R, Wyman C, Rothstein R (2008) Quality control of DNA break metabolism: in the 'end', it's a good thing. *EMBO J* **27:** 581–588

Kane MF, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, Jessup JM, Kolodner R (1997) Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* **57:** 808–811

Kang J, Bronson RT, Xu Y (2002) Targeted disruption of NBS1 reveals its roles in mouse development and DNA repair. *EMBO J* **21:** 1447–1455

Kirchgessner CU, Patil CK, Evans JW, Cuomo CA, Fried LM, Carter T, Oettinger MA, Brown JM (1995) DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science* **267:** 1178–1183

Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, Seeberg E, Lindahl T, Barnes DE (1999) Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc Natl Acad Sci USA* **96:** 13300–13305

Kneitz B, Cohen PE, Avdievich E, Zhu L, Kane MF, Hou Jr H, Kolodner RD, Kucherlapati R, Pollard JW, Edelmann W (2000) MutS homolog 4 localization to meiotic chromosomes is required for chromosome pairing during meiosis in male and female mice. *Genes Dev* **14:** 1085–1097

Krogh BO, Symington LS (2004) Recombination proteins in yeast. *Annu Rev Genet* **38:** 233–271

Kucherlapati M, Yang K, Kuraguchi M, Zhao J, Lia M, Heyer J, Kane MF, Fan K, Russell R, Brown AM, Kneitz B, Edelmann W, Kolodner RD, Lipkin M, Kucherlapati R (2002) Haploinsufficiency of Flap endonuclease (Fen1) leads to rapid tumor progression. *Proc Natl Acad Sci USA* **99:** 9924–9929

Kurimasa A, Ouyang H, Dong LJ, Wang S, Li X, Cordon-Cardo C, Chen DJ, Li GC (1999) Catalytic subunit of DNA-dependent protein kinase: impact on lymphocyte development and tumorigenesis. *Proc Natl Acad Sci USA* **96:** 1403–1408

Kuznetsov S, Pellegrini M, Shuda K, Fernandez-Capetillo O, Liu Y, Martin BK, Burkett S, Southon E, Pati D, Tessarollo L, West SC, Donovan PJ, Nussenzweig A, Sharan SK (2007) RAD51C deficiency in mice results in early prophase I arrest in males and sister chromatid separation at metaphase II in females. *J Cell Biol* **176:** 581–592

Leibeling D, Laspe P, Emmert S (2006) Nucleotide excision repair and cancer. *J Mol Histol* **37:** 225–238

Li L, Salido E, Zhou Y, Bhattacharyya S, Yannone SM, Dunn E, Meneses J, Feeney AJ, Cowan MJ (2005) Targeted disruption of the Artemis murine counterpart results in SCID and defective V(D)J recombination that is partially corrected with bone marrow transplantation. *J Immunol* **174:** 2420–2428

Li Z, Scherer SJ, Ronai D, Iglesias-Ussel MD, Peled JU, Bardwell PD, Zhuang M, Lee K, Martin A, Edelmann W, Scharff MD (2004) Examination of Msh6- and Msh3-deficient mice in class switching reveals overlapping and distinct roles of MutS homologues in antibody diversification. *J Exp Med* **200:** 47–59

Lim DS, Hasty P (1996) A mutation in mouse rad51 results in an early embryonic lethal that is suppressed by a mutation in p53. *Mol Cell Biol* **16:** 7133–7143

Lipkin SM, Moens PB, Wang V, Lenzi M, Shanmugarajah D, Gilgeous A, Thomas J, Cheng J, Touchman JW, Green ED, Schwartzberg P, Collins FS, Cohen PE (2002) Meiotic arrest and aneuploidy in MLH3-deficient mice. *Nat Genet* **31:** 385–390

Liu CY, Flesken-Nikitin A, Li S, Zeng Y, Lee WH (1996) Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev* **10:** 1835–1843

Liu Y, Masson JY, Shah R, O'Regan P, West SC (2004) RAD51C is required for Holliday junction processing in mammalian cells. *Science* **303:** 243–246

Lomonosov M, Anand S, Sangrithi M, Davies R, Venkitaraman AR (2003) Stabilization of stalled DNA replication forks by the BRCA2 breast cancer susceptibility protein. *Genes Dev* **17:** 3017–3022

Ludwig DL, MacInnes MA, Takiguchi Y, Purtymun PE, Henrie M, Flannery M, Meneses J, Pedersen RA, Chen DJ (1998) A murine AP-endonuclease gene-targeted deficiency with post-implantation embryonic progression and ionizing radiation sensitivity. *Mutat Res* **409:** 17–29

Ludwig T, Chapman DL, Papaioannou VE, Efstratiadis A (1997) Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev* **11:** 1226–1241

Luo G, Santoro IM, McDaniel LD, Nishijima I, Mills M, Youssoufian H, Vogel H, Schultz RA, Bradley A (2000) Cancer predisposition caused by elevated mitotic recombination in Bloom mice. *Nat Genet* **26:** 424–429

Luo G, Yao MS, Bender CF, Mills M, Bladl AR, Bradley A, Petrini JH (1999) Disruption of mRad50 causes embryonic stem cell lethality, abnormal embryonic development, and sensitivity to ionizing radiation. *Proc Natl Acad Sci USA* **96:** 7376–7381

Maizels N (2005) Immunoglobulin gene diversification. *Annu Rev Genet* **39:** 23–46

Mak TW, Hakem A, McPherson JP, Shehabeldin A, Zablocki E, Migon E, Duncan GS, Bouchard D, Wakeham A, Cheung A, Karaskova J, Sarosi I, Squire J, Marth J, Hakem R (2000) Brcal required for T cell lineage development but not TCR loci rearrangement. *Nat Immunol* **1:** 77–82

Marsischky GT, Filosi N, Kane MF, Kolodner R (1996) Redundancy of *Saccharomyces cerevisiae* MSH3 and MSH6 in MSH2-dependent mismatch repair. *Genes Dev* **10:** 407–420

McPherson JP, Hande MP, Poonepalli A, Lemmers B, Zablocki E, Migon E, Shehabeldin A, Porras A, Karaskova J, Vukovic B, Squire J, Hakem R (2006) A role for Brca1 in chromosome end maintenance. *Hum Mol Genet* **15:** 831–838

McPherson JP, Lemmers B, Chahwan R, Pamidi A, Migon E, Matysiak-Zablocki E, Moynahan ME, Essers J, Hanada K, Poonepalli A, Sanchez-Sweatman O, Khokha R, Kanaar R, Jasin M, Hande MP, Hakem R (2004a) Involvement of mammalian Mus81 in genome integrity and tumor suppression. *Science* **304:** 1822–1826

McPherson JP, Lemmers B, Hirao A, Hakem A, Abraham J, Migon E, Matysiak-Zablocki E, Tamblyn L, Sanchez-Sweatman O, Khokha R, Squire J, Hande MP, Mak TW, Hakem R (2004b) Collaboration of Brca1 and Chk2 in tumorigenesis. *Genes Dev* **18:** 1144–1153

McWhir J, Selfridge J, Harrison DJ, Squires S, Melton DW (1993) Mice with DNA repair gene (ERCC-1) deficiency have elevated levels of p53, liver nuclear abnormalities and die before weaning. *Nat Genet* **5:** 217–224

Morales M, Theunissen JW, Kim CF, Kitagawa R, Kastan MB, Petrini JH (2005) The Rad50S allele promotes ATM-dependent DNA damage responses and suppresses ATM deficiency: implications for the Mre11 complex as a DNA damage sensor. *Genes Dev* **19:** 3043–3054

Moynahan ME, Chiu JW, Koller BH, Jasin M (1999) Brca1 controls homology-directed DNA repair. *Mol Cell* **4:** 511–518

Moynahan ME, Pierce AJ, Jasin M (2001) BRCA2 is required for homology-directed repair of chromosomal breaks. *Mol Cell* **7:** 263–272

Nakane H, Takeuchi S, Yuba S, Saijo M, Nakatsu Y, Murai H, Nakatsuru Y, Ishikawa T, Hirota S, Kitamura Y, Kato Y, Tsunoda Y, Miyauchi H, Horio T, Tokunaga T, Matsunaga T, Nikaido O, Nishimune Y, Okada S, Okada S, Tanaka H (1995) High incidence of ultraviolet-B-or chemical-carcinogen-induced skin tumours in mice lacking the xeroderma pigmentosum group A gene. *Nature* **377:** 165–168

Nakatsuru Y, Matsukuma S, Nemoto N, Sugano H, Sekiguchi M, Ishikawa T (1993) *O*6-methylguanine–DNA methyltransferase protects against nitrosamine-induced hepatocarcinogenesis. *Proc Natl Acad Sci USA* **90:** 6468–6472

Narod SA, Foulkes WD (2004) BRCA1 and BRCA2: 1994 and beyond. *Nat Rev Cancer* **4:** 665–676

Ng JM, Vermeulen W, van der Horst GT, Bergink S, Sugasawa K, Vrieling H, Hoeijmakers JH (2003) A novel regulation mechanism of DNA repair by damage-induced and RAD23-dependent stabilization of xeroderma pigmentosum group C protein. *Genes Dev* **17:** 1630–1645

Ng JM, Vrieling H, Sugasawa K, Ooms MP, Grootegoed JA, Vreeburg JT, Visser P, Beems RB, Gorgels TG, Hanaoka F, Hoeijmakers JH,

van der Horst GT (2002) Developmental defects and male sterility in mice lacking the ubiquitin-like DNA repair gene mHR23B. *Mol Cell Biol* **22:** 1233–1245

Niida H, Nakanishi M (2006) DNA damage checkpoints in mammals. *Mutagenesis* **21:** 3–9

Nijnik A, Woodbine L, Marchetti C, Dawson S, Lambe T, Liu C, Rodrigues NP, Crockford TL, Cabuy E, Vindigni A, Enver T, Bell JI, Slijepcevic P, Goodnow CC, Jeggo PA, Cornall RJ (2007) DNA repair is limiting for haematopoietic stem cells during ageing. *Nature* **447:** 686–690

Nilsen H, Stamp G, Andersen S, Hrivnak G, Krokan HE, Lindahl T, Barnes DE (2003) Gene-targeted mice lacking the Ung uracil–DNA glycosylase develop B-cell lymphomas. *Oncogene* **22:** 5381–5386

Nussenzweig A, Chen C, da Costa Soares V, Sanchez M, Sokol K, Nussenzweig MC, Li GC (1996) Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature* **382:** 551–555

Nussenzweig A, Sokol K, Burgman P, Li L, Li GC (1997) Hypersensitivity of Ku80-deficient cell lines and mice to DNA damage: the effects of ionizing radiation on growth, survival, and development. *Proc Natl Acad Sci USA* **94:** 13588–13593

O'Driscoll M, Jeggo PA (2006) The role of double-strand break repair—insights from human genetics. *Nat Rev Genet* **7:** 45–54

Ocampo MT, Chaung W, Marenstein DR, Chan MK, Altamirano A, Basu AK, Boorstein RJ, Cunningham RP, Teebor GW (2002) Targeted deletion of mNth1 reveals a novel DNA repair enzyme activity. *Mol Cell Biol* **22:** 6111–6121

Ogrunc M, Sancar A (2003) Identification and characterization of human MUS81-MMS4 structure-specific endonuclease. *J Biol Chem* **278:** 21715–21720

Orii KE, Lee Y, Kondo N, McKinnon PJ (2006) Selective utilization of nonhomologous end-joining and homologous recombination DNA repair pathways during nervous system development. *Proc Natl Acad Sci USA* **103:** 10017–10022

Osman F, Whitby MC (2007) Exploring the roles of Mus81–Eme1/Mms4 at perturbed replication forks. *DNA Repair (Amst)* **6:** 1004–1017

Ouyang H, Nussenzweig A, Kurimasa A, Soares VC, Li X, Cordon-Cardo C, Li W, Cheong N, Nussenzweig M, Iliakis G, Chen DJ, Li GC (1997) Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination *in vivo*. *J Exp Med* **186:** 921–929

Pamidi A, Cardoso R, Hakem A, Matysiak-Zablocki E, Poonepalli A, Tamblyn L, Perez-Ordonez B, Hande MP, Sanchez O, Hakem R (2007) Functional interplay of p53 and Mus81 in DNA damage responses and cancer. *Cancer Res* **67:** 8527–8535

Peltomaki P, Vasen HF (1997) Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. *Gastroenterology* **113:** 1146–1158

Petrini JH, Xiao Y, Weaver DT (1995) DNA ligase I mediates essential functions in mammalian cells. *Mol Cell Biol* **15:** 4303–4308

Pittman DL, Cobb J, Schimenti KJ, Wilson LA, Cooper DM, Brignull E, Handel MA, Schimenti JC (1998) Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmc1, a germline-specific RecA homolog. *Mol Cell* **1:** 697–705

Pittman DL, Schimenti JC (2000) Midgestation lethality in mice deficient for the RecA-related gene, Rad51d/Rad51l3. *Genesis* **26:** 167–173

Prolla TA, Baker SM, Harris AC, Tsao JL, Yao X, Bronner CE, Zheng B, Gordon M, Reneker J, Arnheim N, Shibata D, Bradley A, Liskay RM (1998) Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. *Nat Genet* **18:** 276–279

Puebla-Osorio N, Lacey DB, Alt FW, Zhu C (2006) Early embryonic lethality due to targeted inactivation of DNA ligase III. *Mol Cell Biol* **26:** 3935–3941

Rada C, Ehrenstein MR, Neuberger MS, Milstein C (1998) Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. *Immunity* **9:** 135–141

Reitmair AH, Schmits R, Ewel A, Bapat B, Redston M, Mitri A, Waterhouse P, Mittrucker HW, Wakeham A, Liu B, Thomason A, Griesser H, Gallinger S, Ballhausen WG, Fishel R, Mak TW (1995) MSH2 deficient mice are viable and susceptible to lymphoid tumours. *Nat Genet* **11:** 64–70

Rijkers T, Van Den Ouweland J, Morolli B, Rolink AG, Baarends WM, Van Sloun PP, Lohman PH, Pastink A (1998) Targeted inactivation of mouse RAD52 reduces homologous recombination but not resistance to ionizing radiation. *Mol Cell Biol* **18:** 6423–6429

Ringvoll J, Nordstrand LM, Vagbo CB, Talstad V, Reite K, Aas PA, Lauritzen KH, Liabakk NB, Bjork A, Doughty RW, Falnes PO, Krokan HE, Klungland A (2006) Repair deficient mice reveal mABH2 as the primary oxidative demethylase for repairing 1meA and 3meC lesions in DNA. *EMBO J* **25:** 2189–2198

Rooney S, Sekiguchi J, Whitlow S, Eckersdorff M, Manis JP, Lee C, Ferguson DO, Alt FW (2004) Artemis and p53 cooperate to suppress oncogenic N-myc amplification in progenitor B cells. *Proc Natl Acad Sci USA* **101:** 2410–2415

Rooney S, Sekiguchi J, Zhu C, Cheng HL, Manis J, Whitlow S, DeVido J, Foy D, Chaudhuri J, Lombard D, Alt FW (2002) Leaky Scid phenotype associated with defective V(D)J coding end processing in Artemis-deficient mice. *Mol Cell* **10:** 1379–1390

Sakamoto K, Tominaga Y, Yamauchi K, Nakatsu Y, Sakumi K, Yoshiyama K, Egashira A, Kura S, Yao T, Tsuneyoshi M, Maki H, Nakabeppu Y, Tsuzuki T (2007) MUTYH-null mice are susceptible to spontaneous and oxidative stress induced intestinal tumorigenesis. *Cancer Res* **67:** 6599–6604

Sands AT, Abuin A, Sanchez A, Conti CJ, Bradley A (1995) High susceptibility to ultraviolet-induced carcinogenesis in mice lacking XPC. *Nature* **377:** 162–165

Schmutte C, Sadoff MM, Shim KS, Acharya S, Fishel R (2001) The interaction of DNA mismatch repair proteins with human exonuclease I. *J Biol Chem* **276:** 33011–33018

Sedgwick B, Bates PA, Paik J, Jacobs SC, Lindahl T (2007) Repair of alkylated DNA: recent advances. *DNA Repair (Amst)* **6:** 429–442

Shinohara A, Ogawa H, Ogawa T (1992) Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell* **69:** 457–470

Shinohara M, Shita-Yamaguchi E, Buerstedde JM, Shinagawa H, Ogawa H, Shinohara A (1997) Characterization of the roles of the *Saccharomyces cerevisiae* RAD54 gene and a homologue of RAD54, RDH54/TID1, in mitosis and meiosis. *Genetics* **147:** 1545–1556

Shu Z, Smith S, Wang L, Rice MC, Kmiec EB (1999) Disruption of muREC2/RAD51L1 in mice results in early embryonic lethality which can be partially rescued in a p53(−/−) background. *Mol Cell Biol* **19:** 8686–8693

Smiraldo PG, Gruver AM, Osborn JC, Pittman DL (2005) Extensive chromosomal instability in Rad51d-deficient mouse cells. *Cancer Res* **65:** 2089–2096

Sonoda E, Sasaki MS, Buerstedde JM, Bezzubova O, Shinohara A, Ogawa H, Takata M, Yamaguchi-Iwai Y, Takeda S (1998) Rad51-deficient vertebrate cells accumulate chromosomal breaks prior to cell death. *EMBO J* **17:** 598–608

Su TT (2006) Cellular responses to DNA damage: one signal, multiple choices. *Annu Rev Genet* **40:** 187–208

Subba Rao K (2007) Mechanisms of disease: DNA repair defects and neurological disease. *Nat Clin Pract Neurol* **3:** 162–172

Sugo N, Aratani Y, Nagashima Y, Kubota Y, Koyama H (2000) Neonatal lethality with abnormal neurogenesis in mice deficient in DNA polymerase beta. *EMBO J* **19:** 1397–1404

Sung P, Klein H (2006) Mechanism of homologous recombination: mediators and helicases take on regulatory functions. *Nat Rev Mol Cell Biol* **7:** 739–750

Suzuki A, de la Pompa JL, Hakem R, Elia A, Yoshida R, Mo R, Nishina H, Chuang T, Wakeham A, Itie A, Koo W, Billia P, Ho A, Fukumoto M, Hui CC, Mak TW (1997) Brca2 is required for embryonic cellular proliferation in the mouse. *Genes Dev* **11:** 1242–1252

Symington LS (2002) Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiol Mol Biol Rev* **66:** 630–670, table of contents

Taccioli GE, Amatucci AG, Beamish HJ, Gell D, Xiang XH, Torres Arzayus MI, Priestley A, Jackson SP, Marshak Rothstein A, Jeggo PA, Herrera VL (1998) Targeted disruption of the catalytic subunit of the DNA-PK gene in mice confers severe combined immunodeficiency and radiosensitivity. *Immunity* **9:** 355–366

Takao M, Kanno S, Shiromoto T, Hasegawa R, Ide H, Ikeda S, Sarker AH, Seki S, Xing JZ, Le XC, Weinfeld M, Kobayashi K, Miyazaki J, Muijtjens M, Hoeijmakers JH, van der Horst G, Yasui A (2002) Novel nuclear and mitochondrial glycosylases revealed by

disruption of the mouse Nth1 gene encoding an endonuclease III homolog for repair of thymine glycols. *EMBO J* **21:** 3486–3493

Takata M, Sasaki MS, Sonoda E, Fukushima T, Morrison C, Albala JS, Swagemakers SM, Kanaar R, Thompson LH, Takeda S (2000) The Rad51 paralog Rad51B promotes homologous recombinational repair. *Mol Cell Biol* **20:** 6476–6482

Takata M, Sasaki MS, Tachiiri S, Fukushima T, Sonoda E, Schild D, Thompson LH, Takeda S (2001) Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. *Mol Cell Biol* **21:** 2858–2866

Tauchi H, Kobayashi J, Morishima K, van Gent DC, Shiraishi T, Verkaik NS, vanHeems D, Ito E, Nakamura A, Sonoda E, Takata M, Takeda S, Matsuura S, Komatsu K (2002) Nbs1 is essential for DNA repair by homologous recombination in higher vertebrate cells. *Nature* **420:** 93–98

Taylor AM, Groom A, Byrd PJ (2004) Ataxia–telangiectasia-like disorder (ATLD)-its clinical presentation and molecular basis. *DNA Repair (Amst)* **3:** 1219–1225

Tebbs RS, Flannery ML, Meneses JJ, Hartmann A, Tucker JD, Thompson LH, Cleaver JE, Pedersen RA (1999) Requirement for the Xrcc1 DNA base excision repair gene during early mouse development. *Dev Biol* **208:** 513–529

Theunissen JW, Kaplan MI, Hunt PA, Williams BR, Ferguson DO, Alt FW, Petrini JH (2003) Checkpoint failure and chromosomal instability without lymphomagenesis in Mre11(ATLD1/ATLD1) mice. *Mol Cell* **12:** 1511–1523

Thompson LH, Schild D (2002) Recombinational DNA repair and human disease. *Mutat Res* **509:** 49–78

Thoms KM, Kuschal C, Emmert S (2007) Lessons learned from DNA repair defective syndromes. *Exp Dermatol* **16:** 532–544

Tian M, Jones DA, Smith M, Shinkura R, Alt FW (2004a) Deficiency in the nuclease activity of xeroderma pigmentosum G in mice leads to hypersensitivity to UV irradiation. *Mol Cell Biol* **24:** 2237–2242

Tian M, Shinkura R, Shinkura N, Alt FW (2004b) Growth retardation, early death, and DNA repair defects in mice deficient for the nucleotide excision repair enzyme XPF. *Mol Cell Biol* **24:** 1200–1205

Tishkoff DX, Boerger AL, Bertrand P, Filosi N, Gaida GM, Kane MF, Kolodner RD (1997) Identification and characterization of *Saccharomyces cerevisiae* EXO1, a gene encoding an exonuclease that interacts with MSH2. *Proc Natl Acad Sci USA* **94:** 7487–7492

Treuner K, Helton R, Barlow C (2004) Loss of Rad52 partially rescues tumorigenesis and T-cell maturation in Atm-deficient mice. *Oncogene* **23:** 4655–4661

Truglio JJ, Croteau DL, Van Houten B, Kisker C (2006) Prokaryotic nucleotide excision repair: the UvrABC system. *Chem Rev* **106:** 233–252

Tsuzuki T, Fujii Y, Sakumi K, Tominaga Y, Nakao K, Sekiguchi M, Matsushiro A, Yoshimura Y, Morita T (1996a) Targeted disruption of the Rad51 gene leads to lethality in embryonic mice. *Proc Natl Acad Sci USA* **93:** 6236–6240

Tsuzuki T, Sakumi K, Shiraishi A, Kawate H, Igarashi H, Iwakuma T, Tominaga Y, Zhang S, Shimizu S, Ishikawa T, Nakamura K, Nakano K, Katsuki M, Sekiguchi M (1996b) Targeted disruption of the DNA repair methyltransferase gene renders mice hypersensitive to alkylating agent. *Carcinogenesis* **17:** 1215–1220

van der Horst GT, Meira L, Gorgels TG, de Wit J, Velasco-Miguel S, Richardson JA, Kamp Y, Vreeswijk MP, Smit B, Bootsma D, Hoeijmakers JH, Friedberg EC (2002) UVB radiation-induced cancer predisposition in Cockayne syndrome group A (Csa) mutant mice. *DNA Repair (Amst)* **1:** 143–157

van der Horst GT, van Steeg H, Berg RJ, van Gool AJ, de Wit J, Weeda G, Morreau H, Beems RB, van Kreijl CF, de Gruijl FR, Bootsma D, Hoeijmakers JH (1997) Defective transcription-coupled repair in Cockayne syndrome B mice is associated with skin cancer predisposition. *Cell* **89:** 425–435

Vasen HF, Moslein G, Alonso A, Bernstein I, Bertario L, Blanco I, Burn J, Capella G, Engel C, Frayling I, Friedl W, Hes FJ, Hodgson S, Mecklin JP, Moller P, Nagengast F, Parc Y, Renkonen-Sinisalo L, Sampson JR, Stormorken A *et al* (2007) Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). *J Med Genet* **44:** 353–362

Veigl ML, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, Li GM, Drummond J, Modrich PL, Sedwick WD, Markowitz SD (1998) Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. *Proc Natl Acad Sci USA* **95:** 8698–8702

Weeda G, Donker I, de Wit J, Morreau H, Janssens R, Vissers CJ, Nigg A, van Steeg H, Bootsma D, Hoeijmakers JH (1997) Disruption of mouse ERCC1 results in a novel repair syndrome with growth failure, nuclear abnormalities and senescence. *Curr Biol* **7:** 427–439

Wei K, Clark AB, Wong E, Kane MF, Mazur DJ, Parris T, Kolas NK, Russell R, Hou Jr H, Kneitz B, Yang G, Kunkel TA, Kolodner RD, Cohen PE, Edelmann W (2003) Inactivation of exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. *Genes Dev* **17:** 603–614

Wesoly J, Agarwal S, Sigurdsson S, Bussen W, Van Komen S, Qin J, van Steeg H, van Benthem J, Wassenaar E, Baarends WM, Ghazvini M, Tafel AA, Heath H, Galjart N, Essers J, Grootegoed JA, Arnheim N, Bezzubova O, Buerstedde JM, Sung P *et al* (2006) Differential contributions of mammalian Rad54 paralogs to recombination, DNA damage repair, and meiosis. *Mol Cell Biol* **26:** 976–989

West SC (1997) Processing of recombination intermediates by the RuvABC proteins. *Annu Rev Genet* **31:** 213–244

Wiesendanger M, Kneitz B, Edelmann W, Scharff MD (2000) Somatic hypermutation in MutS homologue (MSH)3-, MSH6-, and MSH3/MSH6-deficient mice reveals a role for the MSH2–MSH6 heterodimer in modulating the base substitution pattern. *J Exp Med* **191:** 579–584

Williams BR, Mirzoeva OK, Morgan WF, Lin J, Dunnick W, Petrini JH (2002) A murine model of Nijmegen breakage syndrome. *Curr Biol* **12:** 648–653

Wilson III DM, Bohr VA (2007) The mechanics of base excision repair, and its relationship to aging and disease. *DNA Repair (Amst)* **6:** 544–559

Woo Y, Wright SM, Maas SA, Alley TL, Caddle LB, Kamdar S, Affourtit J, Foreman O, Akeson EC, Shaffer D, Bronson RT, Morse III HC, Roopenian D, Mills KD (2007) The nonhomologous end joining factor Artemis suppresses multi-tissue tumor formation and prevents loss of heterozygosity. *Oncogene* **26:** 6010–6020

Xiao Y, Weaver DT (1997) Conditional gene targeted deletion by Cre recombinase demonstrates the requirement for the double-strand break repair Mre11 protein in murine embryonic stem cells. *Nucleic Acids Res* **25:** 2985–2991

Xie Y, Yang H, Cunanan C, Okamoto K, Shibata D, Pan J, Barnes DE, Lindahl T, McIlhatton M, Fishel R, Miller JH (2004) Deficiencies in mouse Myh and Ogg1 result in tumor predisposition and G to T mutations in codon 12 of the K-ras oncogene in lung tumors. *Cancer Res* **64:** 3096–3102

Xu X, Qiao W, Linke SP, Cao L, Li WM, Furth PA, Harris CC, Deng CX (2001) Genetic interactions between tumor suppressors Brca1 and p53 in apoptosis, cell cycle and tumorigenesis. *Nat Genet* **28:** 266–271

Xu X, Wagner KU, Larson D, Weaver Z, Li C, Ried T, Hennighausen L, Wynshaw-Boris A, Deng CX (1999) Conditional mutation of Brca1 in mammary epithelial cells results in blunted ductal morphogenesis and tumour formation. *Nat Genet* **22:** 37–43

Yamaguchi-Iwai Y, Sonoda E, Buerstedde JM, Bezzubova O, Morrison C, Takata M, Shinohara A, Takeda S (1998) Homologous recombination, but not DNA repair, is reduced in vertebrate cells deficient in RAD52. *Mol Cell Biol* **18:** 6430–6435

Yamaguchi-Iwai Y, Sonoda E, Sasaki MS, Morrison C, Haraguchi T, Hiraoka Y, Yamashita YM, Yagi T, Takata M, Price C, Kakazu N, Takeda S (1999) Mre11 is essential for the maintenance of chromosomal DNA in vertebrate cells. *EMBO J* **18:** 6619–6629

Yan CT, Kaushal D, Murphy M, Zhang Y, Datta A, Chen C, Monroe B, Mostoslavsky G, Coakley K, Gao Y, Mills KD, Fazeli AP, Tepsuporn S, Hall G, Mulligan R, Fox E, Bronson R, De Girolami U, Lee C, Alt FW (2006) XRCC4 suppresses medulloblastomas with recurrent translocations in p53-deficient mice. *Proc Natl Acad Sci USA* **103:** 7378–7383

Yoon T, Chakrabortty A, Franks R, Valli T, Kiyokawa H, Raychaudhuri P (2005) Tumor-prone phenotype of the DDB2-deficient mice. *Oncogene* **24:** 469–478

Yoshida K, Kondoh G, Matsuda Y, Habu T, Nishimune Y, Morita T (1998) The mouse RecA-like gene Dmc1 is required for homologous chromosome synapsis during meiosis. *Mol Cell* **1:** 707–718

Zha S, Alt FW, Cheng HL, Brush JW, Li G (2007) Defective DNA repair and increased genomic instability in Cernunnos–XLF-deficient murine ES cells. *Proc Natl Acad Sci USA* **104:** 4518–4523

Zhu J, Petersen S, Tessarollo L, Nussenzweig A (2001) Targeted disruption of the Nijmegen breakage syndrome gene NBS1 leads to early embryonic lethality in mice. *Curr Biol* **11:** 105–109